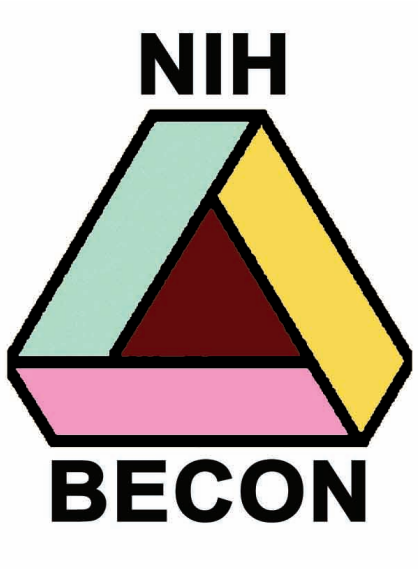
Bioengineering Research Partnerships
Second Annual Grantee Meeting



March 25-26, 2002

Bioengineering Consortium
National Institutes of Health
Bethesda, Maryland

BIOENGINEERING CONSORTIUM

NATIONAL INSTITUTES OF HEALTH
BETHESDA, MD 20892

Welcome to the Second Annual Bioengineering Research Partnership Grantee Meeting.

This February marked the fifth anniversary of the Bioengineering Consortium (BECON) which provides a focus for biomedical engineering research and training activities at the National Institutes of Health (NIH). Active participation by all NIH centers, institutes, and offices and other Federal agencies has facilitated the realization of substantial benefits from the application of engineering, physical, and computational science principles and techniques to address problems in biology and medicine. The importance of this field is reflected by the steady increase in total annual budgets funding for bioengineering research over the past five years and the recent establishment of a National Institute for Biomedical Imaging and Bioengineering at the NIH.

To facilitate the development of the field of bioengineering, the BECON has coordinated trans-NIH initiatives aimed at encouraging and supporting multi-disciplinary and integrative approaches to biomedical research and training. One of the most successful and visible of these research initiatives is the Bioengineering Research Partnership (BRP) Program which was first announced in October 1999. The partnerships that have developed in response to this program are examples of the types of collaboration between the biomedical sciences and allied technical disciplines that can provide significant advances for improving human health. To date, 60 BRP awards have been made for a total investment of about $250 million by fifteen NIH research institutes and centers.

This meeting is the second time that the BRP grantees, BECON members, and NIH institute/center representatives will gatherget together to discuss the research projects, relevant related issues, and the program in general. Your perspectives and suggestions concerning partnership experience, program efficacy, bioengineering research and training needs and directions, and the format for future BRP grantee meetings are solicited. Also, please take this opportunity to meet with your NIH institute/center representative to discuss progress and concerns for your project.

I hope that the BRP Grantee Meeting is valuable and enjoyable to you. All the BECON members and NIH program staff look forward to your participation and comments.

Dr. Jeff Schloss, Chair
Bioengineering Consortium

Bioengineering Research Partnerships
Second Annual Grantee Meeting

Bethesda Hyatt
March 25-26, 2002

FINAL AGENDA

Monday, March 25

Cabinet/Judiciary Room

7:30 AM **Continental Breakfast and Grantee Meetings with NIH Program Officers**

8:30 AM **Welcome** *(Dick Swaja – NIBIB)*

8:45 AM **The BECON and BRP Program** *(Jeff Schloss – BECON)*

9:00 AM **Bioengineering and the NIBIB** *(Donna Dean – NIBIB)*

9:30 AM **Break**

Cabinet/Judiciary & Congressional Rooms

9:45 AM **BRP Grantee Presentations I**
Imaging *(Laurence Clarke – NCI)*, Devices & Rehabilitative Medicine *(Bill Heetderks – NINDS)*

Cabinet/Judiciary Room

11:30 AM **Instructions for Working Lunch**

Cabinet/Judiciary, Old Georgetown & Congressional Rooms

11:45 AM **Working Lunch**

Cabinet/Judiciary Room

1:00 PM **Technology Transfer and Commercialization** *(Michael Weinrich – NICHD)*
Research to Product – A Grantee's Perspective *(Hunter Peckham – CWRU)*
Breaking Through to the Marketplace *(Carmen Catanese – Sarnoff)*
Regulatory Aspects of Medical Devices *(Paul Williams – FDA)*

2:30 PM **Break**

Cabinet/Judiciary Room

3:00 PM **NIH Extramural Research, Review, and Grants Activities** *(Tracy Orr – CSR)*
Extramural Research at the NIH *(Belinda Seto – OER)*
Applications and Grants Management *(Annette Hanopole – NIBIB)*
Bioengineering Review Criteria and Activities *(Eileen Bradley – CSR)*

Fellini's Restaurant

4:30 PM **Networking**

Tuesday, March 26

Cabinet/Judiciary Room

7:30 AM **Continental Breakfast**

8:00 AM **Summary of March 25 Issues** *(Dick Swaja)*

Cabinet/Judiciary & Congressional Rooms

8:15 AM **BRP Grantee Presentations II**
Biomaterials *(Christine Kelley – NIBIB)*, Biomechanics & Informatics *(Lou Quatrano – NICHD)*

9:30 AM **Break**

Cabinet/Judiciary Room

10:00 AM **Bioengineering Issues** *(Dick Swaja)*

11:30 AM **Adjourn**

BECON

BRP Grantee Presentations I

Monday, March 25, 2002
9:45 – 11:30 AM

IMAGING

Cabinet/Judiciary Room

Laurence Clarke (NCI), moderator

1. **Eric Hoffman**
   Image and Model Based Analysis of Lung Disease (NHLBI)
2. **David Vince**
   High Frequency Nonlinear Acoustic Intravascular Imaging (NHLBI)
3. **Henry Halperin**
   Magnetic Resonance Guided Electrophysiology Intervention (NHLBI)
4. **Joseph Izatt**
   Partnership for Research in Optical Coherence Tomography (NIBIB)
5. **Steven Jacques**
   Biomedical Optics for Medical Research And Clinical Care (NIBIB)
6. **H. Grady Rylander**
   Polarization Sensitive Retinal Tomography for Glaucoma (NIBIB)
7. **Bruce Hasegawa**
   Imaging Structure And Function in Small Animals (NIBIB)
8. **Andrew Karellas**
   Digital Mammography High Resolution Flat Panel Imager (NCI)
9. **Clifton Ling / John Humm**
   Multimodality Biological Imaging of Cancer/Tumor Hypoxia (NCI)
10. **Frederic Lizzi**
    Integrated Ultrasonic Systems for Noninvasive Therapy (NCI)
11. **Tuan Vo-Dinh**
    Advanced Multispectral Imaging for Medical Diagnostics (NCI)
12. **Stephen White**
    Cold Neutrons for Biology And Technology (NCRR)
13. **Charles Gilbert**
    Imaging Activity in Visual Cortex At The Cellular Level (NEI)
14. **Sharmila Majumdar**
    Morphological And Functional Musculo-Skeletal Imaging (NIA)
15. **Dennis Discher**
    Bioengineering Research Partnership - Muscular Dystrophy (NIAMS)
16. **Perry Renshaw**
    High Field MR Research in Drug Abuse (NIDA)
17. **Richard Rabbitt**
    Micro-Electric Impedance Spectroscopy of Hair Cells (NIDCD)

DEVICES & REHABILITATIVE MEDICINE

Congressional Room

Bill Heetderks (NINDS), moderator

1. **Manfred Koller**
   Laser Cell Processing for Basic And Clinical Research (NCRR)
2. **Richard Long**
   Blind Pedestrians' Access To Complex Intersections (NEI)
3. **Eliezer Peli**
   Engineering Approaches To Low Vision Rehabilitation (NEI)
18. **Scott Hollister**
    Engineering Joint Scaffolds for Function/Regeneration (NIDCR)
4. **Robert Langer**
   Microchip Drug Delivery System (NIAID)
5. **Thomas Brown**
   Nonlinear Computational Biomechanics of The Hip (NIAMS)
6. **Gregory Stephanopoulos**
   Linking Genomics To Function Via Metabolic Phenotyping (NIDDK)
7. **David Farrell**
   High Tc Susceptometer for Magnetic Measure of Body Iron (NIDDK)
8. **Simon Levine**
   Direct Brain Interface Based on Event Detection in ECOG (NINDS)
9. **Hunter Peckham / Kevin Kilgore**
   Networked Neuroprosthesis (NINDS)
10. **Marc Dichter**
    An Implantable Device To Predict And Prevent Seizures (NINDS)
11. **Houston Wood / Paul Allaire**
    Magnetically Suspended Rotor Blood Pump (NHLBI)
12. **Robert Bartlett / Ronald Hirschl**
    Development of a Total Artificial Lung (NHLBI)
13. **Ronald Hirschl**
    Total Liquid Ventilation: A Bioengineering Partnership (NHLBI)
14. **Edward Crandall**
    Absorption Mechanisms of Peptide/Protein Drugs Via Lung (NHLBI)
15. **William Smith**
    Magscrew TAH Testing thru Pre-Clinical Readiness (NHLBI)
16. **Shimon Weiss**
    Development of Q-Dots As Biological Probes (NIBIB)
17. **Nancy Allbritton**
    Integrated Platform for Chemical Analysis of Live Cells (NIBIB)

BRP Grantee Presentations II

Tuesday, March 26, 2002
8:15 – 9:30 AM

BIOMATERIALS

Cabinet/Judiciary Room

Christine Kelley (NIBIB), moderator

1. **Marcos Intaglietta**
   Bioengineering Design of Artificial Blood (NHLBI)
2. **Alan Snyder**
   Biomedical Applications of Electroactive Polymers (NHLBI)
3. **Svetlana Shabalovskaya**
   Design of Biocompatible Niti (Nitinol) Surfaces (NHLBI)
4. **Buddy Ratner**
   Engineered Cardiac Morphogenesis - Stem Cells And Scaffolds (NHLBI)
5. **Michael Murphy**
   The Design And Fabrication of Novel Micro-Instrument Platforms for Performing Genetic-Based Analyses (NCI)
6. **Rakesh Jain**
   Integrative Biology of Tumor Angiogenesis, Invasion And Metastasis (NCI)
7. **Robert Greenberg**
   Development/Testing of Artificial Retinas for the Blind (NEI)
8. **Thomas Beebe**
   Probing Single-Molecule Neuron-Ligand Pathfinding (NIBIB)
9. **William Shain**
   Brain Prostheses: Tissue Compatibility & Integration (NIBIB)
10. **Shu-Tung Li**
    Type 1 Collagen-Based Nerve Guide for PNS Regeneration (NICHD)
11. **Mark Clemens**
    Engineering Aspects of Liver Support Systems (NIDDK)

BIOMECHANICS & INFORMATICS

Congressional Room

Lou Quatrano (NICHD), moderator

1. **William Huse**
   Drug Discovery of Large-Scale Variant Targets By HTS (NIAID)
2. **Dwayne Westenskow**
   Integration And Visualization of Physiologic Data (NIBIB)
3. **Carlo DeLuca**
   Harnessing Motoneuron Activity: From Lab To Clinic (NICHD)
4. **David Meaney**
   Force Transmission in The Central Nervous System (NICHD)
5. **Laurie Gower**
   Role of Biopolymers And Lipids in Kidney Stone Formation (NIDDK)
6. **Albert Frazier**
   Integrated Sample Preparation for Genomic Analysis in Micro Device Format (NIGMS)
7. **J. Chris Sackellares**
   Bioengineering Research Partnership in Brain Dynamics (NINDS)
8. **Jeffrey Fredberg**
   Micromechanics of Airway Smooth Muscle Cells in Culture (NHLBI)
9. **Jay Humphrey**
   Histo-Mechanics & Biology of Remodeling in Hypertension (NHLBI)
10. **Klaus Ley**
    Biomechanics of Leukocyte Adhesion Molecules (NHLBI)
11. **Thomas Skalak**
    Integrated Control of Vascular Pattern Formation (NHLBI)
12. **Wayne Mitzner**
    New Approach for The Treatment of Asthma (NHLBI)

Attendees

Paul Allaire
Mechanical & Aerospace Engineering
University of Virginia
T: (434) 924-6209
pea@virginia.edu

Nancy Allbritton
Department of Physiology & Biophysics
University of California
T: (949) 824-6493
nlallbri@uci.edu

Thomas Beebe
Department of Chemistry & Biochemistry
University of Delaware
T: (302) 831-1888
beebe@udel.edu

Gary Brittenham
Department of Pediatrics
Columbia University
T: (212) 305-7005
gmb31@columbia.edu

Thomas Brown
College of Medicine
University of Iowa
T: (319) 335-7528
tom-brown@uiowa.edu

Robert Brown
Broncus Technologies, Inc.
rbrown@welch.jhu.edu

Shu Chien
General Campus
University of California - San Diego
T: (858) 534-5195
schien@bioeng.ucsd.edu

Mark Clemens
Department of Biology
University of North Carolina - Charlotte
T: (704) 687-4040
mgclemen@email.uncc.edu

Edward Crandall
School of Medicine
University of Southern California
T: (323) 226-7923
ecrandal@hsc.usc.edu

Chris Danek
Broncus Technologies, Inc.
T: (650) 428-1600 x333
cdanek@broncus.com

Peter Davies
School of Medicine
University of Pennsylvania
T: (215) 573-6813
pfd@pobox.upenn.edu

Carlo Deluca
Neuromuscular Research Center
Boston University
T: (617) 353-9756
cjd@bu.edu

Marc Dichter
Department of Neurology
University of Pennsylvania
T: (215) 349-5166
dichter@mail.med.upenn.edu

Dennis Discher
Biophysical Engineering Lab
University of Pennsylvania
T: (215) 898-4809
discher@seas.upenn.edu

Jim Duncan
Director, Program in Biomedical Engineering
Yale University
T: (203) 785-6322
james.duncan@yale.edu

David Farrell
Physics Department
Case Western Reserve University
T: (216) 368 - 2615
def@po.cwru.edu

Albert Frazier
School of Biomedical Engineering
Univeristy of Utah
T: (404) 894-2030
bruno.frazier@ece.gatech.edu

Jeffrey Fredberg
School of Public Health
Harvard School of Public Health
T: (617) 432-0198
jfredber@hsph.harvard.edu

Charles Gilbert
Graduate And Post Grad Studies
The Rockefeller University
T: (212) 327-7670
gilbert@mail.rockefeller.edu

Laurie Gower
College of Engineering
University of Florida
T: (352) 846-3336
lgowe@mse.ufl.edu

Robert Greenberg
Second Site, LLC
T: (661) 775-3990
bobg@2-sight.com

Henry Halperin
School of Medicine
Johns Hopkins University
T: (410) 955-2412
hhalper@mail.jhmi.edu

Bruce Hasegawa
Physics Research Laboratory
University of California - San Francisco
T: (415) 502-4494
bruceh@itsa.ucsf.edu

Kip Hauch
Scientific Coordinator
University of Washington
T: (206) 543-0289
hauch@u.washington.edu

Ronald Hirschl
Medical School
University of Michigan
T: (313) 764-6846
rhirshl@umich.edu

Eric Hoffman
College of Medicine
University of Iowa
T: (319) 356-1381
eric-hoffman@uiowa.edu

Scott Hollister
College of Engineering
University of Michigan - Ann Arbor
T: (734) 764-9588
scottho@umich.edu

Leroy Hood
Institute for Systems Biology
T: (206) 732-1201
lhood@ystemsbiology.org

John Humm
Medical Physics
Memorial Sloan Kettering Cancer Center
T: (212) 639-7367
hummj@mskcc.org

Jay Humphrey
Department of Biomedical Engineering
Texas A&M University
T: (979) 845-5558
jhumphrey@tamu.edu

William Huse
Novasite Pharmaceuticals, Inc.
T: (858) 597-6811
bhuse@novasite.com

Marcos Intaglietta
Department of Bioengineering
University of California - San Diego
T: (619) 534-4275
mintagli@ucsd.edu

Joseph Izatt
School of Engineering
Case Western Reserve University
T: (216) 844-7928
jai3@po.cwru.edu

Steven Jacques
Department of Dermatology
Oregon Medical Laser Center
T: (503) 216-4092
sjacques@ece.ogi.edu

Rakesh Jain
Department of Radiation Oncology
Massachusettes General Hospital
T: (617) 726-4083
jain@steele.mgh.harvard.edu

Andrew Karellas
Department of Radiology
University of Massachusetts Medical School
T: (508) 856-2069
karellas@umassmed.ummed.edu

Joanne Kelleher
Dept. Chemical Engineering
Massachusettes Institute of Technology
T: (617) 258 0349
jkk@mit.edu

Saeed Khan
Department of Pathology, Immunology and Laboratory Medicine
College of Medicine, University of Florida
T: (352) 392-3574
khan@pathology.ufl.edu

Kevin Kilgore
MetroHealth Medical Center
T: (216)778-3801
klk4@po.cwru.edu

Manfred Koller
Oncosis, Inc.
T: (858) 450-7063
fkoller@oncosis.com

Robert Langer
School of Engineering
Massachusettes Institute of Technology
T: (617) 253-3107
rlanger@mit.edu

Simon Levine
Medical School
University of Michigan - Ann Arbor
T: (734) 936-7170
silevine@umich.edu

Klaus Ley
Cardiovascular Research Center
University of Virginia
T: (434) 924-1722
klausley@virginia.edu

Shu-Tung Li
Collagen Matrix, Inc.
T: (201) 405-1477
cgenmx@aol.com

Brian Litt
Department of Neurology
University of Pennsylvania
T: (215) 349-5166
littb@mail.med.upenn.edu

Frederic Lizzi
Riverside Research Institute
T: (212) 502-1774
lizzi@rrinyc.org

Richard Long
Department of Blind Rehabilitation
Western Michigan University
T: (616) 387-3451
richard.long@wmich.edu

Sharmila Majumdar
School of Medicine
University of California - San Francisco
T: (415) 476-6830
majumdar@clint.ucsf.edu

David Meaney
Department of Bioengineering
University of Pennsylvania
T: (215) 573-3155
meaney@seas.upenn.edu

Wayne Mitzner
Department of Environmental Health Sciences
Johns Hopkins University
T: (410) 614-5446
wmitzner@jhsph.edu

Michael Murphy
Department of Mechanical Engineering
Louisiana State University
T: (225) 578-5921
murphy@lsu.edu

Paul Peckham
Department of Biomedical Engineering
Case Western Reserve University
T: (216) 368-6591
pxp2@po.cwru.edu

Eliezer Peli
Schepens Eye Research Institute
Stephens Eye Research Institute
T: (617) 912-2597
eli@vision.eri.harvard.edu

Richard Price
Department of Biomedical Engineering
University of Virginia
T: (434) 924-0020
rprice@virginia.edu

Richard Rabbitt
Department of Bioengineering
University of Utah
T: (801) 581-6968
r.rabbitt@utah.edu

John Ransom
Director, Flow Cytometry
Novasite Pharmaceuticals
T: (858) 638-8585
jransom@novasite.com

Buddy Ratner
University of Washington
T: (206) 685-1005
ratner@uweb.engr.washington.edu

Perry Renshaw
Brain Imaging Center
McLean Hospital
T: (617) 855-3750
perry@genesis.mclean.org

Michael Rohan
McLean Hospital
mrohan@mclean.harvard.edu

H. Grady Rylander
College of Engineering
University of Texas - Austin
T: (512) 471-1195
rylander@mail.utexas.edu

James Sackellares
College of Medicine
University of Florida
T: (352) 376-1611
sackellares@epilepsy.health.ufl.edu

Svetlana Shabalovskaya
Institute for Physical Research and Technology
Iowa State University
T: (515) 294 1293
shabalov@ameslab.gov

William Shain
Biggs Laboratory
Wadsworth Center
T: (518) 473-3630
shain@wadsworth.org

Chris Sims
Department of Physiology & Biophysics
University of California
T: (949) 824-6493
cesims@uci.edu

Thomas Skalak
Department of Biomedical Engineering
University of Virginia
T: (804) 924-0270
tcs4z@virginia.edu

Larry Sklar
Cancer Research Facility
University of New Mexico
T: (505) 272-6892
lsklar@salud.unm.edu

William Smith
Department of Biomedical Engineering
Cleveland Clinic Foundation
T: (216) 445-9334
wasmith@bme.ri.ccf.org

Alan Snyder
Departments of Surgery And Bioengineering
Penn State University - Hershey Medical Center
T: (717) 531-7068
asnyder@psu.edu

Gregory Stephanopoulos
Department of Chemical Engineering
Massachusetts Institute of Technology
T: (617) 253-4583
gregstep@mit.edu

David Vince
Department of Biomedical Engineering
Cleveland Clinic Foundation
T: (216) 444-1211
vince@bme.ri.ccf.org

Tuan Vo-Dinh
UT-Battelle, LLC-Oak Ridge National Laboratory
T: (615) 574-6249
vodinht@ornl.gov

Shimon Weiss
Department of Chemistry And Biochemistry
Lawrence Berkeley National Laboratory
T: (310) 794-0093
sweiss@chem.ucla.edu

Dwayne Westenskow
Department of Anesthesiology And
Bioengineering
University of Utah
T: (801) 581-2478
dwayne@remi.med.utah.edu

Stephen White
Department of Physiology And Biophysics
University of California - Irvine
T: (949) 824-7122
blanco@helium.biomol.uci.edu

Houston Wood
Mechanical & Aerospace Engineering
University of Virginia
T: (434) 924-6297
hgw9p@cms.mail.virginia.edu

Speakers

Eileen Bradley
NIH Center for Scientific Review (CSR)
T: (301) 435-1179
eb15y@nih.gov

Carmen Catanese
Sarnoff Corporation
T: (609) 734-2000
ccatanese@sarnoff.com

Donna Dean
National Institute of Biomedical Imaging and Bioengineering (NIBIB)
T: (301) 451-6768
deand@nibib.nih.gov

Annette Hanopole
National Institute of Biomedical Imaging and Bioengineering (NIBIB)
T: (301) 451-4780
hanopola@mail.nih.gov

Hunter Peckham
Case Western Reserve University
T: 216-778-3480
pxp2@po.cwru.edu

Belinda Seto
NIH Office of Extramural Research
T: (301) 402-9128
setob@od.nih.gov

Jeff Schloss
National Human Genome Research Institute (NHGRI)
T: (301) 435-5538
js173g@nih.gov

Richard Swaja
National Institute of Biomedical Imaging and Bioengineering (NIBIB)
T: (301) 451-4779
swajar@nibib.nih.gov

Paul Williams
Food and Drug Administration
T: (301) 594-1190
paw@cdrh.fda.gov

BECON Members

Dr. Jeffery A. Schloss, Chair
National Human Genome Research Institute (NHGRI)
T: (301) 435-5538
js173g@nih.gov

Dr. Wendy Baldwin, Ex Officio
NIH Office of Extramural Research (OER)
T: (301) 496-1096
wb4c@nih.gov

Dr. Richard Swaja
National Institute of Biomedical Imaging and Bioengineering (NIBIB)
T: (301) 451-4779
swajar@nibib.nih.gov

Mollie Sourwine
National Institute of Biomedical Imaging and Bioengineering (NIBIB)
T: (301) 451-4775
sourwine@nibib.nih.gov

Dr. Daniel C. Sullivan
National Cancer Institute (NCI)
T: (301) 496-9531
ds274k@nih.gov

Dr. Michael Marron
National Center for Research Resources (NCRR)
T: (301) 435-0755
marronm@ncrr.nih.gov

Dr. Lore Anne McNicol
National Eye Institute (NEI)
T: (301) 496-5301
lm27f@nih.gov

Dr. John T. Watson
National Heart, Lung, and Blood Institute (NHLBI)
T: (301) 435-0513
watsonj@nhlbi.nih.gov

Winifred K. Rossi, M.A.
National Institute on Aging (NIA)
T: (301) 496-6761
wr33a@nih.gov

Dr. Michael Eckardt
National Institute of Alchohol Abuse and Alchoholism (NIAAA)
T: (301) 443-6107
me25t@nih.gov

Dr. Gregory Milman
National Institute of Allergy and Infectious Diseases (NIAID)
T: (301) 496-8666
gm16s@nih.gov

Dr. Richard Morris
National Institute of Allergy and Infectious Diseases (NIAID)
T: (301) 594-7634
rm69e@nih.gov

Dr. Maria Giovanni
National Institute of Allergy and Infectious Diseases (NIAID)
T: (301) 496-1884
mg37u@nih.gov

Dr. James S. Panagis
National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
T: (301) 594-5055
jp149d@nih.gov

Dr. Joan Harmon
National Institute of Biomedical Imaging and Bioengineering (NIBIB)
T: (301) 451-4776
joan_harmon@nih.gov

Dr. Louis A. Quatrano
National Institute of Child Health and Human Development (NICHD)
T: (301) 402-2242
quatranl@hd01.nichd.nih.gov

Dr. Michael Weinrich
National Institute of Child Health and Human Development (NICHD)
T: (301) 402-4201
weinricm@mail.nih.gov

Dr. Lynn Luethke
National Institute on Deafness and Other Communication Disorders (NIDCD)
T: (301) 402-3458
lynn_luethke@nih.gov

Dr. Eleni Kousvelari
National Institute of Dental and Craniofacial Research (NIDCR)
T: (301) 594-2427
ek17W@nih.gov

Dr. Maren Laughlin
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
T: 301-594-8802
laughlinm@extra.niddk.nih.gov

Dr. Thomas G. Aigner
National Institute on Drug Abuse (NIDA)
T: (301) 443-6975
ta17r@nih.gov

Dr. William Suk
National Institute of Environmental Health Sciences (NIEHS)
T: (919) 541-0797
ws22e@nih.gov

Dr. Warren Jones
National Institute of General Medical Sciences (NIGMS)
T: (301) 594-5938
wj5b@nih.gov

Dr. Michael F. Huerta
National Institute of Mental Health (NIMH)
T: (301) 443-3563
mh38f@nih.gov

Dr. William Heetderks
National Institute of Neurological Disorders and Stroke (NINDS)
T: (301) 496-1447
wh7q@nih.gov

Dr. Hilary D. Sigmon
National Institute of Nursing Research (NINR)
T: (301) 594-5970
hs38k@nih.gov

Dr. Merlyn Rodrigues
National Library of Medicine (NLM)
T: 301-496-4621
rodrigM@mail.nlm.nih.gov

Dr. Alex Gorbach
NIH Clincal Center (CC)
T: 301-435-9361
gorbach@ninds.nih.gov

Dr. Eileen Bradley
NIH Center for Scientific Review (CSR)
T: (301) 435-1179
eb15y@nih.gov

Dr. Philip S. Chen
NIH Office of Intramural Research (OIR)
T: (301) 402-3561
pc17w@nih.gov

Dr. Richard Leapman
NIH Office of Research Services (ORS)
T: (301) 496-2599
rl2b@nih.gov

Dr. Michael Viola
Office of Science,
US Department of Energy (DOE)
T: (301) 903-3213
michael.viola@science.doe.gov

Dr. Deborah Crawford
Office of Director,
National Science Foundation (NSF)
T: (703) 292-8003
dcrawfor@nsf.gov

Grantee Summary Reports

PI: **BARTLETT, ROBERT H., M.D.**
2920B Taubman Center
Ann Arbor, MI 48109-0331
T: (313) 936-5822
F: (313) 936-5830
ROBBAR@UMICH.EDU

PROJECT TITLE: Development of a Total Artificial Lung

PARTNERS' NAMES AND AFFILIATIONS:

James Grotberg, Ph.D., M.D.
Biomedical Engineering Department
University of Michigan

Joe Bull, Ph.D.
Biomedical Engineering Department
University of Michigan

GRANTING NIH INSTITUTE/CENTER: Natinoal Heart, Lung and Blood Institute (NHLBI)

ABSTRACT:
The use of mechanical devices ("artificial organs") to replace vital organ function in acute or chronic organ failure has gone from theory to the intensive care bedside in the last 40 years. For 30 of those years our research on artificial organs has been supported by NIH, resulting in devices and techniques now used clinically to treat lung, heart, kidney, and liver failure. One of these techniques (Extracorporeal Life Support, ECMO) can replace lung function for weeks resulting in recovery of otherwise fatal acute lung disease. But ECMO is too complex and invasive to serve as a bridge to lung transplantation. A bridging system is needed because most of the patients listed for lung transplantation die on the waiting list, and because many potential lung donors are not accepted because borderline lung function might prove fatal in the postoperative period without mechanical support. An implantable prosthetic lung, which could function for 3-6 months, would solve both of these problems, just as the ventricular assist device has been applied to cardiac failure and transplantation.

We have applied the expertise of our laboratory to the development of a paracorporeal/ implantable total artificial lung; perfused by the right ventricle and capable of total respiratory support. We have demonstrated safety and efficacy of a prototype design during seven days of implantation in sheep. Based on our preliminary studies demonstrating proof of principle, we propose to design and test a total artificial lung to the point of initial clinical trials. In this proposal, we further intend to establish a new collaboration and partnership between medical researchers and bioengineers who have specific expertise in fluid dynamics, gas transport, artificial organ development, extracorporeal support, and pulmonary physiology to develop and refine a TAL such that it can be implanted successfully as a total lung replacement. This bioengineering project is ideally suited for support by the Bioengineering Research Partnership.

STATUS OF RESEARCH AND PARTNERSHIP:
Our grant for the above project was just recently awarded. We have initiated work on exploring the effect of placement of a low resistance, low compliance conduit between the pulmonary artery and the left atrium upon pulmonary vascular impedance and right ventricular function. We are also developing bench top data and theoretical models of pulmonary vascular compliance which will lead to in-vitro assessment of the requirements for artificial lung compliance.

ISSUES:
We are fortunate to have an already established a bioengineering partnership as part of a separate, prior BRP. We hold conferences involving all members of the partnership, along with trainees, every 2 to 4 weeks. Ongoing research is discussed and data presented at these meetings with vital input contributed from both the bioengineering and clinical investigators. In fact, this BRP proposal to develop a clinically applicable, implantable lung replacement was a product of routine presentations and discussions at our previous BRP meetings which resulted in submission of the current proposal.

We have numerous trainees involved in the partnership including two M.D. research fellows, one medical student, one bioengineering Ph.D. research fellow, 2 bioengineering Ph.D. candidates, and 1 bioengineering masters/undergraduate student. These trainees have been critical to sustaining the cross-fertilization and integration that has been valuable to this partnership.

We have no specific problems with the partnership.

PI: **BEEBE, THOMAS P., JR., PH.D.**
Department of Chemistry & Biochemistry
University of Delaware
Newark, DE 19716
T: (302) 831-1888
beebe@udel.edu

PROJECT TITLE: Probing Single-Molecule Neuron-Ligand Pathfinding

PARTNERS' NAMES AND AFFILIATIONS:

Patrick Tresco, Ph.D.
W.M Keck Center for Tissue Engineering
University of Utah
Salt Lake City, UT 84112
T: (801) 581-8873
patrick.tresco@m.cc.utah.edu

Vladimir Hlady, Ph.D.
Department of Bioengineering
University of Utah
Salt Lake City, UT 84112
T: (801) 581-5042
vladimir.hlady@m.cc.utah.edu

GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke (NINDS)

ABSTRACT:
This research will provide insight into the relationship between biomaterial surfaces and the biology of neurite outgrowth, with the goal of doing so at single-molecule detail, using a battery of quantitative physical measurement and characterization techniques that have never been applied to problems of neurite outgrowth. We hypothesize that the surface density, spatial distribution of ligands, as well as the manner in which ligands are presented on biomaterials (conformationtatus) control bioactivity (axonal outgrowth). Further, we believe that these parameters, in addition to neuronal age, determine the likelihood that a particular neuron will regenerate or grow to establish connections. We hypothesize that this behavior is mediated by changes in membrane adhesiveness for ligands that can be up- or down-regulated in a context-dependent manner. We also predict that this modulation will be different for different extracellular matrix (ECM) ligands, that CNS-derived neurons will adapt to changes in ligand availability differently than peripheral sensory neurons, and that this ability to adapt will decline with neuron age. At least two strategies can be envisioned for bridging substrates. One is a simple biomaterial whose surface is covered with ligand that is permissive for directional neurite outgrowth, and another is a biomaterial with adherent cells used to promote directed neuronal outgrowth. This project seeks to gather information that will be useful in guiding the development of both approaches. We propose to study neurite outgrowth and the dynamics of neuronal membrane adhesiveness and diffusivity of cells derived from different aged hosts grown on well-characterized substrates (artificial materials derivatized with varying densities of specific ligands), as well as more physiologically relevant and complex substrates such as astrocyte monolayers derived from hosts of different ages. Analysis will be conducted using a combination of immunohistochemical, single-molecule, and surface analytical techniques. The specific aims will be approached in a synergistic fashion using the strengths of the different methodologies shared between the three principal two investigators. The methodologies used in single-molecule bond-rupture and single-molecule fluorescence measurements have allowed the direct and highly controlled measurement of protein-ligand, protein-protein, and protein-surface interactions on an individual, molecule-by-molecule basis. Similar ligand-receptor studies will be extended to the study of molecules involved in the guidance of neuron growth cones and axons. This molecular understanding of the balance between specific and non-specific ligand-receptor interactions will be supplemented with microscopy studies (fluorescence and atomic force) of axon development on ligand-modified substrates.

STATUS OF RESEARCH AND PARTNERSHIP:
This BRP was funded in October of 2001. The partnership brings together the expertise of a surface analytical chemist (Beebe) possessing a background in bond-rupture force measurements using the atomic force microscope, surface modification, and state-of-the-art surface analytical techniques, with the expertise of a biophysics-oriented bioengineer (Hlady) possessing a background in protein-surface interactions, evanescent wave optics, time-resolved fluorescence spectroscopy, scanning probe microscopy and a complementary set of state-of-the-art surface analytical techniques, and a neuroscience-oriented bioengineer (Tresco) possessing a background in tissue engineering, nervous system repair and biomaterials.

The three specific aims are to: (1) create and fully characterize model substrates with a controlled pattern and surface density of laminin, fibronectin and related oligopeptides for neuron and astrocyte attachment and axonal growth studies in the two other Aims; (2) Study neurite outgrowth (dynamic bond strength, diffusivity, surface density and bond-rupture force) of dorsal root ganglion neurons on the model substrates, employing single-molecule techniques such as AFM bond-rupture measurements and fluorescence correlation spectroscopy; (3) Study the axonal growth of neurons (dynamic bond strength, diffusivity, surface density and bond-rupture force) on confluent astrocyte monolayers of different ages, using the same methodologies. Underpinning all studies are extensive quantitative surface characterization techniques.

Fluorescence correlation spectroscopy (FCS) is a powerful technique for measuring the diffusion characteristics and concentrations of fluorescent species. The proposed application of FCS by the Hlady group in this project is to probe integrin-receptor diffusion and concentration on distinct portions of the neural cell membranes that are interacting with well-characterized substrates. FCS is well suited to this task, however the nature of the measurement environment requires one to have a well-characterized and optimized FCS device. At Utah, instrument development has been the main push in this portion of the project and is now nearing completion. The development process was recently greatly aided by graduate student Bryon Wright's visit to the lab of Dr. Watt Webb at Cornell University. Dr. Webb's group invented the FCS technique in the early 1970's and they have been in the forefront of the renaissance of the technique that began in the late 1990's. At Delaware, instrument purchase, instrument set-up and development have been the main focus in these initial stages of the project. The PI's group recently moved from Utah to Delaware just as the project was getting underway.

ISSUES:
In the initial stages of the work, the Beebe group at Delaware will be primarily responsible for surface analysis (XPS, TOF-SIMS and AFM) of model surfaces prepared by both the Hlady group at Utah and by the Beebe group itself. This part of the project, mostly that of a supporting role, has begun in earnest. Work at Delaware of a leading role is to prepare biologically functionalized AFM tips and use them to measure single-molecule bond-rupture forces between ligands and integrin receptors, first on fixed, and then on live neurons supported on well characterized surfaces. To this end, the Beebe and Hlady groups have begun to carry out step-wise surface chemical reactions of several varieties as outlined in the proposal. These protocols are being developed to produce AFM tips and glass substrates with patterns of polypeptides initially, and then monolayers of laminin and fibronectin that retain their full bioactivity. The Tresco group has provided expertise in the harvesting and preparation of fixed and live neurons for initial imaging experiments to assess the conditions required for AFM image acquisition.

PI: BERNS, MICHAEL W.
Co-Investigator: Nancy Allbritton
(Dr. Allbritton):
Department of Physiology & Biophysics
Center for Biomedical Engineering
Medical Sciences I, D380
T: (949) 824-6493
nlallbri@uci.edu

PROJECT TITLE: Integrated Platform for Chemical Analysis of Live Cells

PARTNERS' NAMES AND AFFILIATIONS:
Michael Berns[1,3]
Nancy Allbritton[2,6]
Guann-pyng Li[3,4,6]
Mark Bachman[3,4,6]
Vasan Venugopalan[1,5,6]
Christopher Sims[2]

[1]Beckman Laser Institute, [2]Dept. of Physiology & Biophysics, [3]Dept. of Electrical and Computer Engineering, [4]Integrated Nanosystems Research Facility, [5]Dept. of Chemical Engineering and Materials Science, [6]Center for Biomedical Engineering

GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

ABSTRACT:
The overall aim of this partnership is to design, build and test an integrated optical and microfluidics system that will enable the performance of novel biochemical assays in single, living cells. The specific aims of the research are 1) to develop a laser microscope platform for single cell manipulation and analysis, 2) to develop a multipurpose, modular microfluidics chip for single cell analyses, and 3) to develop a broad range of analytes for cell assays. The development process will involve basic studies of the physical mechanisms of laser interactions with cells and polymeric materials used to manufacture the chips, basic engineering of polymer-based microfluidic devices, integration of the microfluidic devices and microscope platform, and further development of novel biochemical assays to be performed with the integrated system.

STATUS OF RESEARCH AND PARTNERSHIP:
During the 2.5 years of the research partnership, significant progress in all three specific aims has been made. An important aspect of the work involves an on-chip method for lysis of a cell and termination of ongoing biochemical reactions with a temporal resolution sufficient for accurate measurements of biologic processes. We have proposed to perform this step with a pulsed laser microbeam that is highly compatible with the polymeric microfluidics devices under development. Analysis of the highly focused pulsed laser microbeam demonstrates that laser-induced plasma formation and cavitation bubble dynamics with subsequent shock wave emission are the primary mechanisms for this process. We have developed a method to measure and visualize the spatial distribution of pulsed laser irradiation within absorbing samples. This is being used to determine the spatial distribution of volumetric energy density deposited within the polymeric device by the pulsed microbeam.

Significant work and accomplishment in the design and manufacture of the microfluidic devices has taken place. In the design of the devices, several industry standard tools have been used to aid in the study of chip design. New software is being developed for chip design and simulation that allows non-experts to accurately design useful devices. Since the development process of these microfluidic devices requires that many prototypes be manufactured, several techniques for rapid development of the prototypes have been evolved which exploit the low cost of processing using poly(dimethylsiloxane) (PDMS) as the polymer for manufacture of the device. This rapid prototyping technology has been transferred to the UCI Integrated Nanosystems Research Facility for general use. To increase the effectiveness of the microfluidic devices, several new schemes for integrating optical and electrical functions are under development. These include integrating electrical lines by inexpensive electroporation techniques from the printed circuit board industry, and optical waveguides manufactured by polymer techniques.

The study of electrophoresis on the polymer microfluidic devices requires a thorough understanding of the surface properties of PDMS. Oxidized PDMS was studied over a period of two weeks as was the effects of exposure to air, aqueous buffer, and base. The electroosmotic mobility has been studied on the PDMS devices and compared with electrophoresis in channels on glass/PDMS hybrid devices. A variety of surface treatments have also been investigated as to their effect on electrophoretic separations performed on the microfabricated devices. Additional work has demonstrated the short-term survival of human tumor cells (Jurkat) within channels of the PDMS/glass devices. Experiments to lyse cells within the microchannel with a shock wave generated by a 5 ns pulsed laser microbeam were successful. The microscope platform has also been engineered with hardware and software to automate or remotely control its operation.

A number of analytes for biochemical assays are under development. New fluorescent labeling and deprotection protocols for the preparation of enzyme substrates to be used in the biochemical assays have been optimized and put into practice. A peptide substrate for protein kinase C fused to a nuclear localization sequence was shown to target the nucleus of cells for a leukemia cell line. Initial testing of electrophoretic separations of analytes based on green fluorescent protein (GFP) has been initiated on chip. Two variants of GFP have been shown to readily separate and can be detected on the microdevice by on their fluorescence.

The active collaboration of the multidisciplinary researchers has been a significant strength in our research effort. Almost every aspect of the above-described work has involved the close interactions among members of the partnership. Concrete examples of this interactive relationship are shared postdoctoral fellows between the investigators, regular attendance of investigators at the lab meetings of fellow investigators, and formal meetings of all the investigators, postdoctoral fellows and students involved in the project. The format of these combined meetings consists of a formal presentation by an individual investigator of work relevant to the partnership followed by a general discussion of issues involved with the research. Attesting to the success of this research program are papers co-authored by various investigators within the partnership.

ISSUES:
none

PI: **BRITTENHAM, GARY M., M.D.**
Professor of Pediatrics and Medicine
Columbia University College of Physicians and Surgeons
Harkness Pavilion, Room HP568
630 West 168th Street
New York, N.Y. 10032-3795
T: 212-305-7005
gmb31@columbia.edu

PROJECT TITLE: High TC susceptometer for magnetic measure of body iron

PARTNERS' NAMES AND AFFILIATIONS:
Case Western Reserve University, Cleveland, OH;
Los Alamos National Laboratory, Los Alamos, NM
Tristan Technologies, Inc., San Diego, CA

GRANTING NIH INSTITUTE/CENTER: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

ABSTRACT:
This Bioengineering Research Partnership will integrate bioengineering, basic science and clinical efforts in the design, development and clinical validation of a high-transition-temperature (high-TC; operating at 77°K) superconducting susceptometer for the direct, non-invasive measurement of hepatic iron stores in patients with iron overload from hereditary hemochromatosis, thalassemia major, sickle cell disease and other disorders. Our laboratories originally proposed that storage iron (ferritin and hemosiderin) could be non-invasively assessed in vivo because of its paramagnetic properties. We subsequently developed low-transition-temperature (low-TC; operating at 4.2°K) superconducting quantum interference device (SQUID) biosusceptometry as a clinical method for the measurement of hepatic iron stores. Non-invasive magnetic measurements of hepatic storage iron in patients with iron overload are quantitatively equivalent to biochemical determinations on tissue obtained by biopsy but the cost and complexity of the low-TC instrument has restricted clinical adoption of the method. Our low-TC susceptometer has three elements which utilize superconductivity: (i) the SQUID, (ii) the field coils that produce a localized steady magnetic field near the liver, and (iii) the detection coils and flux transformer. Recent technological advances make possible replacement of each of these low-TC elements, cooled by liquid helium, with components able to function when cooled by liquid nitrogen. To provide "proof of principle", we have constructed and operated a prototype high-TC susceptometer with (i) a high-TC SQUID, (ii) a NdBFe permanent magnet providing a strong localized magnetic field, and (iii) detection coils and flux transformer fashioned from a high-TC Y1Ba2Cu3O7-d film deposited on a flexible substrate. The Partnership will optimize and integrate these components into a series of liquid nitrogen-cooled clinical devices, and then validate and certify the high-TC susceptometers in studies of adult and pediatric patients. Magnetic studies permit accurate, direct, and repeated measurements of hepatic iron stores not possible with any other method. The development of an affordable, readily usable instrument for the non-invasive measurement of hepatic iron would be a major advance in the diagnosis and management of patients with iron overload that would find immediate and widespread clinical use both in the U.S. and worldwide.

STATUS OF RESEARCH AND PARTNERSHIP:
Active. Since the project was funded on September 30, 2001, rapid progress has been made in manufacturing the custom electronic and cryogenic components for the assembly of the first prototype susceptometer, with contributions from all partners.

ISSUES:

Expectations and Future of the BRP Program
BRP Review and Evaluation Criteria
Commercialization and Publication Issues

PI: **BROWN, THOMAS D.**
Orthopaedic Biomechanics Laboratory
2181 Westlawn
University of Iowa
Iowa City, IA 52242
T: (319) 335-7528
tom-brown@uiowa.edu

PROJECT TITLE: Nonlinear Computational Biomechanics of the Hip

PARTNERS' NAMES AND AFFILIATIONS:
Michael G. Conzemius, DVM, Ph.D., Iowa State University
Robert A. Poggie, Ph.D., Implex Corporation
Richard A. Brand, M.D., John J. Callaghan, M.D., J. Lawrence Marsh, M.D.
Stuart L. Weinstein, M.D., University of Iowa

GRANTING NIH INSTITUTE/CENTER: National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

ABSTRACT:
Disorders of the hip comprise a substantial fraction of current musculoskelatal disease burden. Complex nonlinear mechanical phenomena pervade many aspects of treatment of hip disease and injury, including total hip arthroplasty, intra-articular fractures, osteonecrosis, and developmental dysplasia. While bioengineering capabilities exist - in principle - to quantify key mechanical factors influencing treatment outcomes in these areas, contemporary clinical decision making still rests almost entirely on subjective empirical experience. This Bioengineering Research Partnership (BRP) brings together the capabilities of an experienced computational biomechanics research group, four senior orthopaedic hip surgeons, a veterinary research orthopaedist, and an industry-based materials scientist, in order to advance the state of the art in biomechanically-grounded management of disorders and injuries of the human hip. The central focus of the research Partnership lies in applying nonlinear finite element formulations to address as-yet-unquantified mechanical phenomena that are clinically recognized as being crucial to patient outcome. Building on previous and ongoing finite element work, new computational formulations will be developed to tackle nonlinearities currently limiting the accuracy of numerical simulations in five clinically important areas of hip surgery. The first two areas involve leading complications of total hip arthroplasty. First, as regards abrasive wear of polyethylene, we propose to incorporate local directionality of femoral head counterface motion in computing wear rates with a sliding-distance-coupled contact finite element formulation. Second, as regards dislocation, we propose to introduce soft tissue tethering into a large-displacement sliding contact model of resistance to dislocation. The third area involves intra-articular fractures of the acetabulum: estimating residual cartilage contact stress elevations accompanying attempts at surgical restoration of articular surface congruity. The fourth area involves osteonecrosis: computationally characterizing a new animal model (the emu) which unlike previous animal models progresses to human-like femoral head collapse, and using that model for in-vivo testing of computationally optimized placement of a novel head-preserving implant device. The fifth application area involves surgical management of developmental hip dysplasia: using novel mesh pre-processing techniques to quantify improvements of intra-articular contact stress achieved by pelvic osteotomies. This Partnership will bring together a critical mass of engineers and surgeons, to achieve clinically-grounded advances in nonlinear numerical simulations of surgery of the hip.

STATUS OF RESEARCH AND PARTNERSHIP:

Our scientific work continues to move along very well. Voxel-based contact finite element analysis is now operational. Energy-based assessment of fracture severity has turned out to be tedious in terms of person-hours required for image analysis. But, the data have convincingly established proof of concept, and the project won this year's Selvik Award from the European Orthopaedic Research Society. Our technique for patient-specific preprocessing to zone imprecisely reduced central acetabular fractures is now operational, and early results have been presented at a national meeting. Most of the computer code is operational for direction-dependent THA wear, although we still have not completed the fixturing for validation testing, pending deciding on the best technique to produce controlled counterface scratches. Our work with the emu model has gone very well: we now have a reliable cryo-insult probe which produces segmental lesions, and which progress to collapse. We have augmented the emu work to now include automated image analysis histology, to quantify the infarction zone. Our research partners remain enthusiastically committed to the collaboration.

ISSUES:

No problems at this point. Our concern last year about Implex possibly being restructured, due to affiliation with or purchase by Zimmer, has turned out to be unwarranted. Although the "market" for Hedrocel implants indeed has heated up substantially now that Zimmer is involved, this has turned out to work to our advantage, rather than disadvantage, because Implex was able to procure additional resources to invest in its Hedrocel fabrication facilities. Our design of Hedrocel implant rods for the emu model, to all appearances, worked satisfactorily on the first try, so now it is just a matter of Implex producing additional implants for us, as we expand our emu study series.

PI: **CLEMENS, MARK G.**
Department of Biology
University of North Carolina at Charlotte
9201 University City Blvd
Charlotte NC 28223
T: 704-687-4040
mgclemen@email.uncc.edu

PROJECT TITLE: Engineering aspects of liver support systems

PARTNERS' NAMES AND AFFILIATIONS:
Robin Coger (Mechanical Engineering), Charles Lee (Mechanical Engineering), Jian Zhang (Biology), Laura Schrum (Biology)

GRANTING NIH INSTITUTE/CENTER: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

ABSTRACT:
In spite of many advances in liver transplant surgery, an increasing number of patients with terminal liver disease are dying while awaiting transplants. Consequently, further advances in the storage of donor livers, as well as alternative replacement options and mechanisms for supporting liver function while awaiting a donor liver are needed. A very promising area of research and development is in the development of engineered solutions to the problems of liver support for either natural donor organs or bioartificial livers. However, efforts undertaken within a single discipline are hampered by the complexity of both the engineering and biological aspects of such projects. This proposal constitutes a partnership between bioengineers, biologists and a liver transplant surgeon with the goal of combining their expertise to devise improved methods of liver support via bioartificial livers and improved preservation of donor livers via machine perfusion preservation (MPP). The partnership encompasses three inter-related projects. The first project focuses on delivery of oxygen and other nutrients to the cells in in vitro systems such as the bioartificial liver. The approach involves the modification of the support matrix to facilitate enhanced mass transport. The second project addresses the hypothesis that improved bioartificial liver function can be attained by providing a more physiological combination of cell types in the support device. Specifically, we will investigate the relationship between Kupffer cells and hepatocytes in maintaining prolonged hepatic-specific function in culture. The final project focuses on development of methods for optimization of microvascular perfusion in machine- perfused livers. This project uses a combination of intravital microscopy and mathematical modeling. In all of the projects, engineering and biological approaches, as well as clinical experience, are combined to address focused, clinically relevant problems. Moreover, the unique environment that supports the partnership will maximize the potential for success in this interdisciplinary approach and provide an avenue for potential clinical application of laboratory advances.

STATUS OF RESEARCH AND PARTNERSHIP:
Funding was initiated on this project of Sept. 30, 2001. Since then, we have made tangible progress on Project 3 and have initiated experiments related to Projects 1 and 2.

Project 1: The first year of Project 1 is focused on SA#4. This choice was made, in part, because it enabled us to involve our Chapel Hill collaborators in the project within Year 1. We have already designed the physical setup needed to complete this aim, and have planned the necessary set of experiments. In addition to acquiring the BAL prototypes, this step included setting up a dynamic

flow non-BAL system in which a defined cellular space is sandwiched between 2 convective flows. This step was necessary for 2 reasons: a) the BAL prototype is expensive and not expected to be re-usable b) the BAL prototype is likely to experience fouling, and c) the non-BAL system is better suited for testing how the dimensions of the annulus of the BAL prototype should be adjusted. At this juncture we are waiting for the arrival of ordered supplies - including the O2 meters and the pumping system.

We expect the first set of experiments to commence at the beginning of March. Through them we will determine if implementing the enhanced collagen ECM within the BAL improves the overall function of hepatocytes, as compared to the non-enhanced system. We will also evaluate how the functional results of the homogeneously enhanced system compare to at least 1 example of a modified enhanced system.

Project 2: We have begun evaluating critical tissue culture conditions for these studies. Thus far, we have successfully used elutriation to isolate Kupffer cells and hepatocytes, and we have determined a culture medium able to support both cell types in the same culture. In the coming months we expect to establish the micropatterning technique for the defined co-culture of hepatocytes and Kupffer cells and evaluate the morphology, viability, and function, of both cells in the micropatterned systems.

Project 3: Currently, we have shown that after 24 hrs of machine perfusion at 4°C, there is stasis of fluorescent-labeled red blood cells. In addition, experiments with fluorescently-labeled albumin in the vasculature show regions of no flow, interstitial edema, and normal flow. Regions of no-flow and interstitial edema correspond to regions with increased red cell stasis. These results would indicate blockage in the sinusoids and a possible cause of damage to the liver. Histology shows that the majority of endothelial cells have become rounded, but direct evidence of endothelial cells restricting red cell movement is not yet available. We initiated experiments utilizing DiI-acetylated low density lipoprotein (DiI-acLDL) to fluorescently mark endothelial cells to monitor during 24hr machine perfusion. Since DiI-acLDL fluoresces at a wavelength different from that of the red cell, if rounded endothelial cells are the cause of red cell stasis, this set of experiments should provide direct evidence as to the cause of the blockage. In addition, we have constructed a temperature controlled microshear chamber and have begun studies of the effect of shear stress and hypothermic temperatures on endothelial cell structure. We have built a device to monitor light intensity of our fluorescent microscopes in order to normalize our fluorescence signals for quantification. In addition, we have begun to calibrate NADH and rhodamine 123 fluorescence signals for monitoring in vivo metabolic state of hepatocytes.

ISSUES:

The major issues that confront the partnership at this time are:

1. Completion of the acquisition and setup of new equipment (especially imaging setup) for the project.
2. Completion of the establishment of procedures for assessment of the maintenance of hepatocyte-specific function in Bioartificial Liver prototypes.
3. Establishment of liver cell cultures on micropatterned surfaces.

PI: **CRANDALL, EDWARD D.**
Department of Medicine
University of Southern California Keck School of Medicine
IRD 606, 2020 Zonal Avenue
Los Angeles, CA 90033
T: (323) 226-7593
ecrandal@usc.edu

PROJECT TITLE: Absorption mechanisms of peptide/protein drugs via lung

PARTNERS' NAMES AND AFFILIATIONS:
Vincent H.L. Lee, Ph.D. [Project 2 leader]
Department of Pharmaceutical Sciences, USC School of Pharmacy
Wei-Chiang Shen, Ph.D. [Project 3 leader]
Department of Pharmaceutical Sciences, USC School of Pharmacy
Kwang-Jin Kim, Ph.D. [Project 4 leader]
Department of Medicine, USC Keck School of Medicine

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
Our long-term goal is to elucidate the mechanisms for absorption of peptide and protein drugs across the alveolar epithelium that offers most of the surface area available for drug absorption in the lung airspaces. We will utilize primary cultured rat alveolar epithelial cell monolayers as an in vitro model to investigate absorption mechanisms of the alveolar epithelium and delineate possible ways to enhance transport rates of peptide/protein drugs. Various di-/tri-peptides, granulocyte-colony stimulating factor (G-CSF, 18.8 kDa) and human growth hormone (hGH, 22 kDa) will be used as model drugs. We propose to [1] delineate the mechanisms and pathways (i.e., paracellular diffusion, fluid-phase transcytosis, receptor-mediated and/or adsorptive transcytosis) for transepithelial transport of model protein drugs across the alveolar epithelial barrier, [2] investigate the mechanisms underlying stimulation of protein drug absorption via transcytosis across the alveolar epithelial barrier, [3] investigate how di-/tri-peptide drugs are absorbed across the alveolar epithelium, and [4] determine the stimulatory effects of physicochemical variables (e.g., the hyperosmotic gradient imposed by dissolution of peptide/protein drug in the thin alveolar lining fluid, acidic pH of the lining fluid, and controlled release mechanisms that may be related to a specific formulation (e.g., large porous carrier particles) of the peptide/protein drugs) on alveolar epithelial absorption of protein and di-/tri-peptide drugs. Through the collaborative investigation of pulmonary protein/peptide drug absorption among four different laboratories utilizing experimental approaches spanning from cell biology/physiology to bio(chemical)engineering, we will provide new information on how alveolar epithelium handles peptide/protein drugs. These studies will be useful for devising new drug formulation and delivery methodologies to improve the bioavailability of poorly absorbed peptide/protein drugs via the alveolar epithelial barrier.

STATUS OF RESEARCH AND PARTNERSHIP:
Projects 1 and 4 (Elucidation of transcytotic pathways / Effects of biophysical parameters (e.g., pH, osmolality) and soluble factors (e.g., hormones) on protein transport across alveolar epithelium): We are studying the mechanisms and regulation of IgG transport across primary cultured rat alveolar epithelial cell monolayers (RAECM) grown on tissue culture-treated polycarbonate filters. Based on the preliminary evidence that IgG is net absorbed across the RACEM via saturable process, we further studied if alveolar epithelial IgG transport is mediated by FcRn as in neonatal intestine.

We designed primers based on published sequence of rat FcRn gene and performed RT-PCR. The fragment obtained from such molecular approach yielded a product whose sequence is 100% identical to that predicted from rat sequence. Treatment of RAECM with 100 nM dexamethasone for two days led to ~50% reduction in FcRn mRNA level by Northern analysis, concomitant with a decrease in IgG flux in the apical-to-basolateral (but not opposite) direction. These results are consistent with a thesis that net absorption of IgG across rat alveolar epithelium occurs via FcRn-mediated transcytosis which appears to be regulable by glucocorticoids as in neonatal intestinal epithelium.

Project 2 (Studies of alveolar epithelial di-peptide transporters): Our laboratories previously reported that RNA extracted from RAECM shows the presence of PepT2 message, as evidenced by alveolar epithelial cells on both days 1 and 5. The intensity of GAPDH is similar across specimens, assuring equal loading of samples. The RT-PCR product obtained from alveolar epithelial cells matches fully the predicted sequence for PepT2. In addition to this proton-dependent oligopeptide transporters, we recently found that RAECM also express processes for specifically transporting neutral and cationic amino acids (i.e., ATB0,+). We confirmed by uptake studies that ATB0,+ is localized in predominantly apical side of cell monolayers. Neutral (except for proline) and cationic amino acids inhibited the uptake of 3H-labeled arginine, consistent with known characteristics of ATB0,+. Kinetic analyses indicated that the maximal velocity and Michaelis-Menten constant are 33.32 ± 2.12 pmol/mg protein/15 min and 0.504 ± 0.114 mM, respectively. We recently cloned rat ATB0,+ which predicts a protein comprised of 640 amino acids. These results are consistent with a thesis that rat pneumocytes express the amino acid transport system B0,+.

Project 3 (Transferrin receptor (TfR)-mediated modulation of protein transport across alveolar epithelium): Transferrin (Tf) was found to be transported across RAECM, by TfR specific transcytosis, in the apical-to-basolateral direction in keratinocyte growth factor (KGF) and brefeldin-A (BFA) treated monolayers. KGF treated monolayers that did not receive BFA treatment demonstrated relatively minor TfR-specific Tf transport capabilities. Control monolayers (no KGF treatment) did not demonstrate any detectable TfR-specific Tf transport with or without the addition of BFA. Next, an insulin-transferrin (In-Tf) conjugate was tested for the ability to be transported across RAECM by TfR-specific transcytosis. In-Tf transport properties were similar to Tf, with BFA enhancing the TfR-mediated apical-to-basolateral transcytosis of Tf in KGF treated monolayers.

ISSUES:

1. Project leaders participate in monthly meetings to develop overall strategy, develop further collaborations, and plan optimal directions for the four projects. Quarterly scientific meetings of all personnel involved in the projects are held, where each group presents its latest research findings.
2. Monthly seminar series sponsored by the project leaders is held under the auspices of the USC Center for Drug Design/Delivery. Recent speakers include: Phil Factor, M.D. (Associate Professor of Medicine, Northwestern University, 3/9/01), Franck C. Szoka, Ph.D.(Professor of Pharmaceutical Sciences, UCSF, 3/16/01), Jindrich (Henry) Kopecek, Ph.D. (Professor of Pharmaceutical Sciences, University of Utah, 4/13/01), Jo Rae Wright, Ph.D. (Professor of Cell Biology, Duke University, 4/20/01), Leaf Huang (Professor of Pharmaceutics, University of Pittsburgh, 12/13/01), Kenneth Thummel, Ph.D. (Professor of Pharmaceutics, University of Washington, 3/29/02).
3. We are planning to establish an in-situ/in-vivo small animal facility for pulmonary drug delivery studies to complement the mechanistic studies described above using our in vitro model.

PI: **DAVIES, PETER F**
University of Pennsylvania
T: (215) 573 6813
pfd@pobox.upenn.edu

PROJECT TITLE: Cell and Molecular Studies in Cardiovascular Engineering

PARTNERS' NAMES AND AFFILIATIONS:
Childrens Hospital of Philadelphia (CHOP)

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
This is a multi-investigator proposal from a single campus that addresses fundamental bioengineering mechanisms in cardiovascular cells and their preclinical application in vivo and ex vivo. Most of the program is physically located at Penn's Institute for Medicine and Engineering (IME), which was established to connect Medical School and Engineering School scientists working at the interface between biomedicine and the engineering, physical, and computational sciences. Our approach is to understand the cell and molecular mechanisms by which the local physical and chemical environment regulates cardiovascular cell and tissue physiology and to combine this with testing and implementation of engineering principles in tissues and experimental animals. This is particularly important in the cardiovascular system where the biomechanical, structural, and chemical environments are spatially complex. The program addresses both hypothesis-driven and design-driven experimental approaches in varying proportion. The BRP investigators, a mix of biomedically-trained and engineering-trained faculty, share a strong commitment to interdisciplinary research and represent a community of multidisciplinary scholars.

STATUS OF RESEARCH AND PARTNERSHIP:
Grant began 9/01.
Integrative partnership with CHOP on same campus
In the first year, basic studies of mechanotransduction hypothesis-testing, design-driven measurements of forces in and around cells (eg FRET measurements in cell adhesion), and the development of novel viscoelastic substrates are being established. In tandem,the critical role of mechanotransduction in three pre-clinical studies is investigated. In the first focus, ex vivo arterial remodeling experiments of arteries and veins to develop replacement autologous grafts is a difficult but extraordinarily important objective. It combines complex hemodynamic engineering with basic mechanisms of vascular remodeling. Preliminary results are promising for long-term maintenance of arterial structure-function and a controlled transition of vein to artery. The causes of heart valve calcification are not understood. Both aortic and pulmonary valves are exposed to (different) complex mechanical and flow environments. In the second pre-clinical focus, the involvement of the Human Heart Valve Registry at Childrens Hospital, is a great resource for the program. The well developed pig valve and coarctation models are excellent for investigations of pathologic mechanisms involving tissue biomechanics and cell dysfunction including the use of advanced molecular biology techniques. The introduction of valves into ex-vivo flow circuitry interacts naturally with the engineering measurements involved in graft remodeling. Site-specific therapy to repair intracranial aneurysms by the use of growth factor-coated platinum coils and adenovirus combines intricate surgical protocols (neurosurgery) with materials science, polymer coatings, mass transport of vectors, and vascular wall biology.

ISSUES: No negative issues to date

PI : **DE LUCA, CARLO, J.**
Boston University, NeuroMuscular Research Center,
19 Deerfield Street, 4th Flr. Boston, MA 02215
T: (617) 353-9757
cjd@bu.edu

PROJECT TITLE: Harnessing Motoneuron Activity: From Lab to Clinic

PARTNERS' NAMES AND AFFILIATIONS:

Dr. Hamid Nawab, Boston University
Dr Rick Roark, New York Medical Center
Dr. Zeynep Erim, Boston University
Dr. Mario Manto, Free University of Brussels

GRANTING NIH INSTITUTE/CENTER: National Institute for Child Health and Human Development (NICHD), National Center for Medical Rehabilitation Research (NCMRR)

ABSTRACT:
We propose to develop an automatic system for decomposing the electromyographic (EMG) signal into the constituent action potentials corresponding to the firing of individual motor units activated by motoneurons. The system will be an outgrowth of our existing rudimentary system, which over the past 20 years has enabled us to perform various novel investigations that have provided a variety of new insights into motor control. However, the current system suffers from many limitations, which curtail its usefulness as a research tool, and has never been useful as a Clinical Tool. The new system will have a dramatically enhanced performance: 1) decomposition time for typical contractions will be decreased from dozens of hours to a few minutes, 2) the automatic decomposition accuracy will be increased from 60 % to 95% - with provisions for assisted editing to reach 100% accuracy, 3) it will be able to decompose signals from dynamic as well as static contractions (which is a current limitation), 4) it will weigh less than 10 kg, and will have a notebook computer configuration, and 5) most importantly the decomposition algorithms will be completely rewritten using a newly developed knowledge-based Artificial Intelligence language blackboard platform developed by us. This platform has been used successfully to decompose polyphonic signals and radar spread spectrum signals having a complexity comparable to that of the EMG signal. The proposal is composed of 5 projects. The first and dominant project will be Design Driven. It describes the design and development of the new system, which has at its heart, a Knowledge-Based algorithms for decomposing the signals. The other four projects will be hypotheses-based and will address basic science questions and clinical applications that will reveal the utility of the new system. These projects will also be used to test and improve the evolving design of the new system. Project 2 will address the modifications, which occur in the firing of motor units as a function of Aging. Project 3 will address the phenomenon of motor unit substitution, which will be useful in Ergonomics work environments and in the Rehabilitation of patients with Peripheral Nerve Injury and Spinal Cord Injury. Projects 4 and 5 are two Clinical Studies. Project 4 will explore the use of quantified neuromotor activity for developing prognostic indicators for determining denervation and re-enervation of Paralyzed Laryngeal Muscles. Project 5 will study patients with acute ataxia following a Cerebellar Stroke to explore the manifestation of CNS disorders in the firing characteristics of the motoneurons.

STATUS OF RESEARCH PARTNERSHIP:

Project # 1 - Decomposition of the EMG Signal -- There are two main components to this project: the software and the hardware.

Software - The development of the EMG signal decomposition software is on schedule. In FY2 we have improved the implementation of the front-end signal processing software and we have developed a knowledge-based process for improving the performance of our EMG decomposition system, whose overall design is based on the IPUS architecture from the field of Artificial Intelligence. As reported last year, the new front-end algorithms are capable of detecting and classifying multi-channel motor unit firings at speeds two or more orders of magnitude greater than that of the previous algorithms, thus approaching real-time performance. This year, we have improved the front-end's accuracy performance (percentage of motor units that are correctly identified) from an average of 65% to an average of 75% without sacrificing its computational efficiency. Meanwhile the error rate (percentage of detections that are incorrectly identified) for the front-end decreased from an average of 5% to an average of 2%. We have also developed and implemented a prototype version of a knowledge-based process for improving upon the results obtained from the front-end. We have found that this process is able to correctly identify many of the detections that are either misclassified or left unclassified by the front-end. It does so by making use of IPI-based predictions of the locations of MU firings and by verifying those predictions using a correlation-based analysis of the signal data. Furthermore, as compared to the front-end, the execution time of this knowledge-based process is essentially negligible. In the coming year, we shall investigate other aspects of knowledge-based processing for improving the system's accuracy performance to the desired 95%+ level without the need for human intervention and without sacrificing real-time performance.

Hardware -- The development of the system hardware is on schedule. A final design of the prototype of the

signal processing electronics hardware was successfully evaluated. A full set of documentation is being prepared. The next phase of hardware development is the fabrication and integration of the electronic hardware within a custom enclosure. The data acquisition and signal management was developed with a modular, two component, computer system consisting of a laptop PC connected via a fast Ethernet link to a Panel PC with video display and data acquisition system hardware. This configuration provides both the high-speed capabilities required to perform online decomposition analysis, and the specified small form factor. An "alpha version" software package for controlling the low level-hardware and the User Interfaces was written. The alpha version is fully functional and provides the user with the flexibility to interact with the system, to create test protocols and to control the data acquisition hardware. Subsequent versions will refine the User Interface to provide seamless data collection, signal processing, and display of results.

Project # 2- Aging -- In FY1 we reported that experimental protocol had been refined and the experimental set-up had been finalized. Data collection had begun and some EMG signal decomposition was performed. In FY2 the remaining 5 young healthy subjects were recruited and tested, thus completing data collection for the proposed set of 10 controls. In addition, 6 sedentary elderly subjects were recruited, medically screened and tested. We are in the process of recruiting the last four. We have used the old decomposition system to laboriously decompose the EMG signal of 5 subjects from each age group. Preliminary results show differences in the recruitment, the mean firing rate, and firing variability of motor units when comparing young to elderly subjects. Recently we have started to process the newly recorded data using an alpha version of the new decomposition system.

Project # 3 - Fatigue -- In FY2 we began the third project of the program. Eleven (11) healthy subjects were recruited and tested. All tests were performed on the First Dorsal Interosseous muscle of the dominant hand according to the fatigue-generating protocol described in the proposal. Intramuscular EMG signals and the force patterns were recorded. The signals from eight subjects were decomposed and qualitatively analyzed using the old decomposition system. Preliminary results from 4 subjects have been presented at the Society for Neuroscience Meeting in San Diego. For the remaining three subjects decomposition using an alpha version of the new system was performed and data analysis is currently under way.

Partnership Collaboration - Dr. Nawab's group and the NeuroMuscular Research Center group have formal two-hour bi-weekly meetings to review progress and plan future activities. We have been in contact with our other two partners, Dr. Roark in New York and Dr. Manto in Brussels, Belgium. Although, their projects do not begin until next year, we have already begun discussions and exchanges of personnel. Dr. Roark has visited Boston on two occasions and has already made several suggestions on the specifications of the new decomposition system. A student from Boston has spent a year in Dr. Manto's laboratory in Brussels as a Fulbright Fellow and has now returned.
Information Dissemination - During the past year we have published the following abstracts and papers:

Abstracts:

Adam A, De Luca CJ. Control of motor units during submaximal fatiguing contractions: Abstract # 609, XVIIIth Congress of the ISB, Zurich, 2001.

Adam A, Jain A, Voigt HF, De Luca CJ. Control of Motor Units During Submaximal Fatiguing Contractions in Humans Hand Muscle: Abstract # 168.16, Society for Neuroscience, San Diego 2001

De Luca CJ, Gonzalez-Cueto JA, Bonato P. Motor Unit Recruitment and Reciprocal Inhibition Decrease, Submitted for ISEK Conference 2002

De Luca CJ, Nawab SH, Adam A, Wotiz R, Hochstein L, Gilmore LD, Roark R, Manto M. Precision Decomposition II for EMG Signals. Submitted for ISEK Conference 2002

Erim Z, Burke DT, De Luca CJ. Effects of aging on the control of motor units: Abstract # 677, XVIIIth Congress of the ISB, Zurich, 2001

Erim Z, Burke DT, De Luca CJ. Effects of Aging on Motor Unit Firing Behavior in Different Muscles: Society for Neuroscience, San Diego 2001

Papers:

De Luca CJ, Erim Z. Common Drive in Motor Units of a Synergistic Pair; Journal of Neurophysiology Vol 87: 2002 (in press)

Hochstein L, Nawab SH, Wotiz R. An AI-Based Software Architecture for a Biomedical Application, submitted to SCI 2002

Nawab SH, Wotiz R, Hochstein L, De Luca CJ. Improved Decomposition of Intramuscular EMG Signals, submitted to SCI 2002

ISSUES:
The collaboration within our BRP is has progressed smoothly and productively.

PI: **DICHTER, MARC, M.D., PH.D.**
Department of Neurology
HUP, 3 W. Gates
3400 Spruce Street
Philadelphia, PA 19104
T: (215) 349-5166
dichter@mail.med.upenn.edu

PROJECT TITLE: An Implantable Device to Predict and Prevent Seizures

PARTNERS' NAMES AND AFFILIATIONS:
Brian Litt, M.D. (Neurology and Bioengineering),
Peter Crino, M.D., Ph.D. (Neurology and Neuroscience)
Diego Contreras M.D., Ph.D. (Neuroscience)
Douglas Coulter, Ph.D. (CHOP and Neuroscience)
Lief Finkel, M.D., Ph.D. (Bioengineering)
George Vachtsevanos, Ph.D. (Electrical and Computer Engineering, Georgia Tech)

GRANTING NIH INSTITUTE/CENTER: National Institute for Neurological Disorders and Stroke (NINDS)

ABSTRACT:
Epilepsy affects approximately 1-1.5% of the population. Medications help control seizures in about 60-70% of patients but 30-40% cannot be controlled by current medical or surgical treatment. In this proposal an ensemble of investigators from the University of Pennsylvania, Georgia Institute of Technology (GIT), and Children's Hospital of Philadelphia are engaged in a 5-10 year effort to create a novel therapy for refractory epilepsy: an implantable device capable of predicting epileptic seizures prior to electrical onset and triggering intervention to prevent their clinical expression. The research partnership has three major thrusts: (1) Seizure Prediction: Developing and refining algorithms capable of predicting seizures hours to minutes prior to electrical and clinical onset. These algorithms are based upon signals obtained from implanted biosensors in adults, children and animal models of human epilepsy, (2) Mechanisms of Ictogenesis: Unraveling the neurophysiologic, neuronal network, cellular and molecular, mechanisms underlying the preictal (preseizure) changes identified by these algorithms through in-vitro and in-vivo investigations in the laboratory and clinical settings. Experimental observations will be incorporated into computer simulations of these mechanisms to facilitate development of better prediction and intervention strategies, and (3) Therapeutics: Developing interventions aimed at specific points in the "ictogenic" process, including electrical stimulation and drug infusion, to disrupt the cascade of events leading to seizures while preserving normal brain function. We anticipate that this research partnership will spin off commercially viable intellectual property including: implantable biosensors, miniaturized electrical stimulation and bioinfusion hardware, stimulation paradigms, and customized software/ hardware interfaces for signal acquisition, processing and controlling therapeutic intervention. The ultimate goal of this effort is to bring this technology to clinical trials in humans, and within 10 years to see marketing of an implantable closed-loop seizure prediction and prevention device that will improve the quality of life of individuals with epilepsy.

STATUS OF RESEARCH AND PARTNERSHIP:
Experiments are currently being performed in each of the partnership laboratories. Rats and mice with chronic epilepsy are being produced and seizure prediction algorithms are being implemented and examined. Animal models are being examined with EEG (field potential) and multiunit

recordings. Areas of brain involved in ictogenesis are being identified for molecular studies and computational models are being developed. Brain stimulation protocols are being devised to try to abort seizures.

ISSUES:

1. Technology transfer
2. Working at multiple sites, in different cities
3. Budget cuts and further funding

PI: **FRAZIER, A. BRUNO**
Georgia Institute of Technology, School of Biomedical Engineering,
Microelectronics Research Center, 777 Atlantic Drive, Atlanta, GA 30332
T: (404) 894-2030
F: (404) 894-4700
Bruno.Frazier@ece.gatech.edu

PROJECT TITLE: Integrated Sample Preparation For Genomic Analysis in Micro Device Format

PARTNER'S NAMES AND AFFILIATIONS:

AUSTIN, ROBERT H.	ANDERS, JAMES P.	SWEDBERG, SALLY A.
Princeton University	University of Virginia	Genetics and Proteomics
Department of Physics	Department of Chemistry	ThermoFinnigan, Inc

GRANTING NIH INSTITUTE/CENTER: National Institute of Environmental Health Sciences (NIEHS)

ABSTRACT:
The overall goal of the proposed research is to produce an integrated sample preparation micro-analytical device for preparing blood samples for genomic analyses. The strategy is to develop a front-end micro sample preparation system, µ-SPS, for use as a research tool with the flexibility to be integrated with a number of downstream genetic analysis platforms, i.e. either sequencing or genotyping. The µ-SPS is composed of three main microcompartments including: 1.) Sample introduction, combined with cell sorting and selection. 2.) Cell lysis, recovery of the nucleic acid material of choice (e.g. DNA or mRNA), and sample clean up via solid phase extraction or affinity capture. 3.) Elution of the material to an amplification µ-compartment, and subsequent amplification (e.g. via PCR or rtPCR). Studies of the µ-compartment prototypes are paralleled by an investigation of techniques for integrating the µ- compartments into a monolithic and hybrid µ-SPS.

STATUS OF RESEARCH AND PARTNERSHIP:
Aim 1: Sample Introduction, Cell Sorting, Selection and Lysis:
A. Sample Introduction and Cell Sorting: The Frazier group is working on a micro-compartment that integrates the sample introduction, cell manipulation, cell sorting, concentration and lysing operations. Two primary methods are being investigated for cell manipulation, namely dielectrophoresis and acoustic waves (i.e. bulk waves and flexural plate waves). Designs have been fabricated and tested for both methods. The end result of the studies was that cells can be manipulated in bulk and as single cells using both approaches. The primary differences in the approaches is in the complexity of fabrication and operation. Acoustic waves require the use of piezoelectric materials to sustain the waves generated by an external power source connected to an on-chip interdigitated electrode structure. Both the fabrication and operation of the acoustic devices is complex when compared to the alternative, dielectrophoresis. Dielectrophoresis in combination with off-chip control of the fluid flow rate allows for precise control of the cells in batch and single cell formats. Additionally, dielectrophoresis is components are easily fabricated and do not require special materials of construction. Therefore, future systems will utilize dielectrophoresis as the primary mechanism for cell manipulation. Upstream fractionation and concentration of the whole blood into cellular components has been achieved using field flow fractionation (FFF) technology. FFF technology will perform the cell concentration operations leading up to cell lysis. Cell manipulation via dielectrophoresis and cell concentration via FFF are currently being coupled with the integrated impedance spectroscopy detection system for cell signaturing and sorting.
B. Cell Lysis, and Continuous DNA Pre-filtration: One of the great promises of microfabrication technologies when applied to biology is the potential to extract and analyze the contents of a single cell. The contents of that cell include the chromatin containing the DNA, the RNA components, organelles in the cell and the proteins in the cytoplasm. This is of course an enormous and difficult task which we are far from accomplishing at present. However, some basic steps have been taken towards this goal and the Austin group present some approaches towards the fractionation, lysis, trapping and purification of single cell chromosomes using the techniques of diffusive mixing and dielectrophoretic trapping. First, we have been developing novel methods to sort cells using advanced concepts in microfluidics and nanomagnetics. Next, we move on to examining cells that have been selected. While the ultimate challenge to the single cell project is a human eukaryotic cell, at this stage of work it is more reasonable to work with prokaryotic cells with their single chromosome, well-defined genome of manageable length and relatively simple cellular content. We chose to start this work with E. coli bacterial cells because they are relatively easy to grow and they have been studied extensively compared to other cells. We have been working with two different strains of E. coli, the "wild" type (PS2124 (RP437)) and a green fluorescent protein (GFP)-expressing one (PS2164). Over-expression of GFP allowed us to easily visualize the cells in epi-fluorescence and determine when the cell has been lysed, and potentially allows us to track the protein content (at least the GFP part of protein content) separately from the genomic content of the cell. Lysis of the cells was achieved by mixing water with an osmotically stressed cell (aspheroblast). Once the cell is lysed

we then transport the cell contents into a region where the chromosome of the cell containing the genome can be separated from the other components of the cell. We have utilized the technology of dielectrophoretic trapping at low frequencies to separate the chromosome from the lysis debris.

Aim 2: Recovery of the Nucleic Acid Material, and Sample Clean-up:

Miniaturized Solid Phase Extraction: The Lander's group effort to define a miniaturized system for solid phase extraction (SPE)-based isolation of DNA on the microliter scale have begun to crystallize on two fronts. First, the use of a silica-based adsorption system appears to be feasible approach based on success with silica beads inserted into microchips. Second, the success with silica-based adsorption led us to explore the use of sol-gel matrices, a polymerizable crystalline solution of silica, as potential solid phase for microchip-based DNA extraction. The Lander's group has shown that silica beads inserted into a microchip can be effective for extraction of DNA but that problems with longevity exist. This is due to the fact that, with repeated use, the packed beads reorganize and occlude the outlet. This problem has been resolved by "gluing" the packed silica beads into place in the microchip chamber using sol-gels. Under these conditions, DNA can be selectively eluted in a small volume and the reproducibility of DNA extraction (both run-to-run and chip-to-chip) are reasonably good. It is, perhaps, the sol-gels themselves that hold the most potential for DNA extraction. The key here is the porosity of the polymerized gel matrix which, based on the sol-gel literature, should be trivial to control. However, in reality it is anything but. After changing many of the variables in the sol-gel recipe, we have found a TMOS (tetramethylorthosilicate)-based system that appears to allow for effective DNA extraction. Perhaps most important, we find that the DNA extracted with most solid phases is PCR-amplifiable – this includes TMOS but does not include TEOS. We currently have the first results involving DNA extraction of human genomic DNA (from whole cells) on TMOS-filled microchips. Efforts will continue in this vein while starting to address issues associated with both microchip substrates used (glass thus far but looking to start using some of the substrates defined by the Frazier lab) and integration of the SPE process with PCR on the same microdevice. It is noteworthy that if the sol-gel work continues with positive results, there may be no need to pursue the use of bound peptide nucleic acids for solid phase affinity-based extraction (SPE) of DNA from crude mixtures, as suggested in the original proposal.

Aim 3: Amplification of Nucleic Acid Material:

The method we are championing for on-chip amplification of DNA is IR-PCR. This uses an inexpensive infrared heat source for the accurate control of sample temperature during PCR. We have progressed beyond the ultra-fast temperature cycling already demonstrated on several fronts. We have defined the passivation chemistry required for IR-mediated amplification in glass microchips as well as having defined the passivation chemistry required for separation of neat PCR products in glass microchips. We have defined a number of potentially effective dynamic coatings that may also be used in glass microchip PCR and demonstrated its utility towards human genomic DNA. We explored a number of IR sources, defining a broadband Tungsten lamp as ideal for IR-mediated PCR (not yet published) and defined a new method (UV-induced) for glass-glass microchip bonding. Finally, we have tested a simplistic design for direct injection of on-chip amplified DNA coupled to rapid on-chip separation. Now that the SPE project is well on its way and Frazier's group has defined new candidate substrates, we anticipate significant progress in this area in year 3.

Aim 4: Integration of Independent Prototype Micro Compartments into a Hybrid µ-SPS:

Identification and development of a common materials and fabrication technology base for the compartments of the genomic sample preparation system has been a high priority topic for each of the 3 BRP team meetings during the past year. The consortium will use the common technology base for two main purposes. First, the technology base will provide the BRP team access to a powerful fabrication base for producing prototypes of the micro compartments for characterization. Secondly, to build the infrastructure and pathway to an integrated genomic sample preparation system with all compartments integrated. The technology base needs to flexible in order to allow for a wide range of material choices and operating conditions (ph range, surface coatings, optical interfacing) as required to accommodate the specific needs of each compartment. Additionally, the technology base needs to provide an avenue for integrating electrical (detection) components, off-chip to on-chip fluid interconnects, and other mechanical functionality (i.e., pumps and valves). The BRP team has identified a strategic route to accomplish Aim 4, including a process flow for micro compartment integration; the key fabrication technologies (e.g., injection molding, stereolithography, hot embossing, micro screen printing, micro electro forming) required to fabricate the µ-SPS, and most promising materials (e.g. high molecular weight polyethylene, polycarbonate, polyimide) to be evaluated. All of the necessary equipment and processing infrastructure will be available through Georgia Tech resources beginning year 3.

ISSUES:

Primary issues are the logistics of integrating the technologies developed from the participating laboratories into a common format for the total analysis system. We will address this issue by adding a 'roving' research scientist into the program to aid with dissemination of the base fabrication technologies developed at GaTech to the other sites.

PI: **FREDBERG, JEFFREY**
Harvard School of Public Health
665 Huntington Ave
Boston, MA 02115
T: (617) 432 0198
fredber@hsph.harvard.edu

PROJECT TITLE: Micromechanics of airway smooth muscle cells in culture

PARTNERS' NAMES AND AFFILIATIONS:
Univ.ersity Barcelona
Dalhousie University

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
Acute narrowing of the airway lumen in asthma is driven by myosin motors that exert their mechanical effects within a cytoskeletal scaffolding that is both deformable and in a continuous state of remodeling. The mechanical properties of that scaffolding are not well defined. This BRP application describes a multi-discliplinary design-directed bioengineering project to fill that gap of knowledge. We propose to develop a micromechanical technology to measure the rheological properties of adherent living airway smooth muscle cells in culture, and the time-course of mechanical changes that occur in response to contractile stimuli or after genetic manipulation of cytoskeletal proteins. Ligand-coated ferromagnetic microbeads are bound to the cytoskeleton, and oscillatory mechanical torques are then applied to the bead by a sinusoidally-varying external magnetic field. Resulting oscillatory bead motions deform the cell, and can be determined by measuring changes of the remanent magnetic field due to bead rotations or, alternatively, by direct observation of oscillatory bead displacements using light microscopy; these are complementary detection methods each with special advantages. This technology becomes, in effect, a micro-rheometry system that can probe - in cell culture conditions - contractile responses and underlying cellular rate processes over time scales as short as tens of milliseconds to as long as hundreds of seconds. Thus, it measures mechanical properties of cells using deformation times (and stress magnitudes) that span the physiological range. We propose to develop this technology and then use it to test the hypothesis that the contractile response of human airway smooth muscle cells in culture is attenuated by overexpression of heat shock protein 27 (HSP27) dominant negative mutants. This hypothesis bears upon a question whose importance has been identified only recently, namely, the stability of the cytoskeleton of the airway smooth muscle cell and the role of CSK stability in airway narrowing in asthma.

STATUS OF RESEARCH AND PARTNERSHIP:
Research is progressing on schedule and several publications have appeared already in the literature. Using magnetic bead twisting over a wide range of oscillatory frequencies we have established a remarkable glass-like behavior of the cytoskeleton, and shown that this finding is confirmed when the cells are probed by an independent method, atomic force microscopy. So far, the BRP partners have met for four two-day meetings that have been intense, mutually beneficial and highly productive.

ISSUES:
The issues that we have encountered are all highly positive. The BRP granting mechanism has allowed us to pursue avenues of investigation and to facilitate important collaborations that would otherwise have been most difficult to accomplish.

PI: **GILBERT, CHARLES**
The Rockefeller University
1230 York Avenue
New York, NY 10021-6399
212 327-7670
gilbert@rockefeller.edu

PROJECT TITLE: Imaging activity in visual cortex at the cellular level

PARTNERS' NAMES AND AFFILIATIONS:
Winfried Denk, Max-Planck-Institute, Heidelberg
Roger Tsien, UCSD

GRANTING NIH INSTITUTE/CENTER: Nationonal Eye Institute (NEI)

ABSTRACT:
We are combining advances in optics, molecular probes and gene therapy techniques to monitor neural activity in the visual cortex of awake, behaving animals, with single cell resolution. Two-photon imaging makes it possible to visualize fluorescent cells lying several hundred microns under the cortical surface and to minimize the photodynamic damage to the cells. Fluorescent proteins allow one to visualize the details of cell morphology, and their fluorescence can be linked to measures of neural activity. Adenoviral vectors will be developed to insert genetic constructs that code for these proteins into the genome of cortical cells, allowing one to label large numbers of cells with a sparse distribution and with minimal damage to the cells. We will merge the instrumentation for imaging with the set up for animal recording and behavior, insert constructs into viruses, and adapt the 2-photon microscope for monitoring activity tied to visual stimuli and animal behavior. Being able to record from the same identified cells over an extended period allows one to study experience-dependent changes in the functional properties and morphology of cells. The approach will have a wide range of applications, including the study of morphological changes in cells, the biophysics of neuronal integration, the neural basis of learning and higher order cognitive function, and patterns of gene expression in the intact brain.

STATUS OF RESEARCH AND PARTNERSHIP:
We have had progress on three fronts: developing a new fast imaging system, obtaining new genetically encoded probes for signaling activity, and doing imaging in awake, behaving monkeys.

Fast scan 2-photon imaging. To use 2-photon imaging for monitoring activity of single neurons, we had to adapt the technology for higher temporal resolution. The development of the optics required joining together a resonance scanning head with a second stage to move the scanned area to a number of discrete positions around the microscopic field. The second stage has incorporated in it an X-Y-Z stage for moving the microscope objective, while maintaining alignment with the scanning laser beam. We are writing software to use this for biometric mapping-reconstructing imaged structures in 3-dimensions. We are incorporating additional elements into the scanning system, including vibration isolation and an X-Y gantry for increased flexibility in positioning the scanning head.

Imaging in awake, behaving macaque monkeys. Part of the challenge of this project is to have a stable window in the skull through which one can visualize the brain without damaging the cortex. We designed an imaging chamber for doing optical imaging in awake behaving macaque monkeys. The bottom of the chamber contains a thick glass coverslip, and the cortical surface is further protected

by a transparent artificial dura. Resecting the original dura opens up an avenue for infection, so the chamber has to be monitored on a daily basis for signs of exudate, overgrowth of connective tissue, and swelling or adhesions. We have maintained the chamber in one macaque monkey in this manner for approximately 6 months. We obtained good quality intrinsic signal optical images, suggesting that the cortex is in a good physiological state. To image in the awake animal also requires interfacing our software for behavioral control of the animal, stimulus generation and imaging. We have now successfully integrated all the software components for intrinsic signal optical imaging, and anticipate little difficulty in doing so for the 2-photon imaging.

Genetically encoded fluorescent probes. A third area is the development of genetically encoded fluorophores that are sensitive to neural activity. The variety and quality of probes has greatly improved over the last year, and we have obtained several for testing. The utility of the probes has been improved due to improved functionality under normal conditions in vivo. Also, in addition to sensors of calcium, additional probes have been developed that monitor other aspects of neuronal activity. We are now incorporating these probes into adenoviral vectors.

ISSUES:
The technical challenges we are confronting include maintaining the stability of the preparation for imaging, maintaining the physiological state of the exposed area of the brain, developing probes that provide robust signals related to neuronal activity, and developing vectors for delivery of genetically encoded probes that allow expression of the genes for sufficiently long periods of time.

PI: **GOWER, LAURIE B., PH.D.**
Assistant Professor
Department of Materials Science & Engineering
University of Florida
Gainesville, FL 32611-6400
lgowe@mse.ufl.edu

Co-PI: Saeed R. Khan, Ph.D.
Professor; Director of Experimental Pathology
University of Florida
College of Medicine
khan@pathology.ufl.edu

PROJECT TITLE: Role of Biopolymers and Lipids in Kidney Stone Formation

PARTNERS' NAMES AND AFFILIATIONS:
Daniel Talham
Richard Dickinson
Hassan El-Shall
Yakov Rabinovich

GRANTING NIH INSTITUTE/CENTER: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

ABSTRACT:
The objective of the proposed bioengineering research partnership (BRP), located at the University of Florida, is to examine two key issues relevant to urolithiasis; 1) the effects of acidic biopolymers and lipid membranes on nucleation, growth and aggregation of calcium oxalate (CaOx) crystals in an artificial urinary environment; and 2) the injurious effects of a liquid-phase mineral precursor on tubular epithelial cells grown in culture. With regard to 1), many investigators have examined the promotory and inhibitory effects of acidic glycoproteins on crystal growth and aggregation. Our work differs in that a primary focus will be to investigate the relevance of a recently discovered polymer-induced liquid-precursor (PILP) process to pathological biomineralization. The PILP process generates non-equilibrium crystal morphologies which exhibit features similar to crystals found in kidney stones, such as for example, stratified spherulites. Mineral films and coatings are also deposited by the process, and repetitive depositions might lead to concentrically laminated structures, such as those commonly observed in composite stones. In addition, the interfacial aspects of this liquid-liquid phase separation process lead to a pronounced aggregation tendency of crystals. Lastly, we hypothesize that the presence of this cementatious mineral precursor in the urinary tract could influence the attachment and retention of crystals to renal epithelial cells; or the highly ionic precursor phase could cause cell injury or death, leading to the release of modulatory factors or membrane fragments, which could promote heterogeneous nucleation and/or aggregation of crystals. The proposed work consists of 10 Specific Aims which fall under four topical areas: crystal-macromolecule, crystal-crystal, crystal-lipid, and crystal-cell interactions. The bioengineering techniques to be used include measurement of interparticle forces by Atomic Force Microscopy, measurement of long-range interactions between submicron CaOx particles and mimetic lipid membranes with an optical trap force transducer, and nucleation of crystals and PILP phase on mimetic lipid membranes using Langmuir monolayers. This 5-year project will enable us to assess the relevance of the PILP process to pathological calcification, as well as to perform a comparative analysis with the more traditional concepts pertaining to the role of lipids and acidic biopolymers in stone formation, and will contribute to the development of bioengineering techniques that are new to the field of stone research. The long-range clinical goal of this BRP is to provide a more effective means of diagnosis, treatment, and long-term prevention of renal calculi.

STATUS OF RESEARCH AND PARTNERSHIP:
The progress that has been made in the first year has been primarily a continuation of some of the feasibility studies presented in the proposal. In particular, the work in Gower's lab has focused

on developing a better synthetic model for mimicking the reaction environment of urine within kidney tubules, as well as synchrotron x-ray scattering studies of mineral precursor films deposited onto amphiphilic monolayers. El-Shall's lab has expanded from the initial studies on the influence of polymeric steric repulsive forces on reducing crystal aggregation (this work now accepted for publication in Journal of Colloid and Interface Science), to examining the effect of similar mimetic proteins on crystal nucleation and growth. Results of this work have been submitted as an abstract for presentation to the annual meeting of the "ROCK" in Orlando May 2002. Due to the convenient location of this conference, several other members of the BRP team plan to submit abstracts and attend. Talham's lab has installed the Brewster Angle Microscope requested in the proposal, and is examining the early stages of crystal nucleation grown under mimetic lipid monolayers. Rabinovich's work has focused on AFM measurement of particle-particle interactive forces in the presence of urinary constituents, and the more recent results are being used to explain previous preliminary data and a paper is being prepared for publication, including several collaborators from the research team. Another task is to measure forces between crystals and cultured renal epithelial cells, in which Dr. Khan's lab is assisting with the biological aspects of that study, and is also examining the fate of the cells subjected to the liquid-phase mineral precursor prepared in Gower's lab. Dickinson's lab has been developing the techniques for examining particle interaction forces with mimetic lipid membranes using Langmuir-Blodgett deposition techniques combined with the evanescent wave, light scattering/3D-optical trapping technique, which enables measurement of picoNewton forces of interaction. Overall, the collaborative expertise of our team has already proven to be a strong asset to the research, and we anticipate this BRP grant will prove very successful.

ISSUES:

It is somewhat difficult to find post-docs and graduate students with a background suitable for this interdisciplinary work, so there is a substantial hurdle to overcome in the early stages of the project (which is probably the case for many BRPs). We have used biweekly meetings to provide an overview of the field from each project leader's viewpoint to assist in training the newcomers to the project. These meetings have helped in integrating research efforts and mutual planning of tasks. Drs. El-Shall and Rabinovich have hired two graduate students this year since they could not fill the post-doc position. They will continue searching for a person who is qualified for the post-doc position and hope to have it filled by the second year.

PI: **GREENBERG, ROBERT J.**
Second Sight, LLC
28460 Avenue Stanford, Suite 200
Valencia, California 91355
T: (661) 775-3990
bob@2-sight.com

PROJECT TITLE: Development/Testing of Artificial Retinas for the Blind

PARTNERS: University of Southern California; Alfred E. Mann Foundation; Massachusetts Eye and Ear Infirmary; Massachusetts Institute of Technology; Illinois Institute of Technology; North Carolina State University; University of California, Los Angeles; Bionic Technologies

GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

ABSTRACT:
Our research for this partnership grant is to develop a long-term implantable retinal stimulator for patients blinded by outer retinal degenerations. Using technologies developed by the Alfred E. Mann group of companies over the past 30 years for implantable stimulators, we are developing a chronic retinal stimulator and associated external hardware for use both in research and as a clinical device.

In order to achieve this goal, several areas of research are still needed. In this bioengineering research partnership, academia collaborates with industry to accomplish the basic research necessary to make a chronic retinal prosthesis a reality. Areas of basic research that we focus on include:

* Electrode geometry and electrode material selection
* Surgical attachment of the retinal implant
* Low power electronic circuit design
* Hermetic packaging

Each of these areas needs additional research for the creation of an optimal chronic retinal prosthesis which will enable persons blinded by outer retinal degenerations to regain the most important loss they have suffered--the loss of mobility. The aim of this five-year proposal is to complete the design and manufacture of a retinal prosthesis and associated external hardware and test it chronically in animals, so that an investigational device application can be made to the FDA in preparation for a clinical trial.

STATUS OF RESEARCH AND PARTNERSHIP:
The partnership continues to make great strides in its second year.

Most of the new candidate materials which were under test last year did not survive long term stimulation. However, one new material appears to function much better than the prior industry standard, platinum, and has been under test for six months so far. This new material will allow much denser electrode arrays which will hopefully provide finer resolution for a retinal prosthesis.

Improved retinal tacks for securing the device to the back of the eye have been developed by several of our partners. A vibratory retinal tack inserter has also been developed which may aid in the insertion of retinal tacks.

Chronic animal studies in dogs with wireless implants are underway at USC. Over 15 active wireless

implants have been implanted to date. In the histology examined so far, electrical stimulation does not appear to have affected any of the retinal cells underneath the arrays for periods of up to three months of stimulation. Longer periods of stimulation have not yet been tested.

More advanced devices with higher electrode counts are under development. Testing of the fourth generation integrated circuit (the 'brain' of the implant) is complete and it is functioning well. A fifth generation integrated circuit is currently being designed. Development of packaging and electrodes for these advanced devices also continues to advance with several approaches under test.

ISSUES:
One of our partners moved their entire lab across the country (from Johns Hopkins to USC) to be closer to our group. This has greatly increased our team's productivity, but managing some remote sites and increasing communication across the US remains challenging. The entire group meets together in person twice a year which is always a fabulous experience. Everyone leaves invigorated (in part because there is visible progress by the team members after 6 months). We use emails and conference calls in between meetings and continue to search for ways to better 'close the loop.'

PI: **HALPERIN, HENRY R.**
Johns Hopkins University
Blalock 524; 600 N Wolfe Street
Baltimore, MD 21287
T: (410) 955-2412
F: (410) 955-0223
hhalper@jhmi.edu

PROJECT TITLE: Magnetic Resonance Guided Electrophysiology Intervention

PARTNERS' NAMES AND AFFILIATIONS:
Johns Hopkins University (Medicine, Radiology, Biomedical Engineering)
Guy Clatterbaugh: Johns Hopkins Applied Physics Laboratory
Erez Nevo: Robin Medical, Incorportated
Steve Sagon: Bard Electrophysiology, Incorporated
Mark Adams: Irvine Biomedical Incorporated

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

ABSTRACT:
Ventricular tachyarrhythmias and atrial fibrillation are the most important arrhythmias affecting patients. They are the most frequently encountered tachycardias, account for the most morbidity and mortality, and despite much progress, remain therapeutic challenges. Invasive electrical studies of the heart (electrophysiologic studies) are often used in the diagnosis and therapy of arrhythmias, and many arrhythmias can be cured by selective destruction of critical electrical pathways with radiofrequency (RF) catheter ablation. A major limitation in studying arrhythmias in patients, however, is the lack of ability to accurately correlate anatomical and electrical information. Anatomy is derived from x-ray images, which are two-dimensional and have substantial anatomic ambiguity. Another major limitation is the lack of ability to visualize ablated areas of myocardium during catheter ablation procedures, making it difficult to confirm the presence of ablated lesions in the desired locations. We have developed ways of combining the anatomic information from magnetic resonance imaging (MRI), with electrophysiologic testing and catheter ablation.

We hypothesize that magnetic resonance imaging, with transesophageal receivers, intracardiac receivers and MRI-compatible (non-magnetic) electrode catheters, can (1) provide accurate navigation of catheters without radiation, (2) provide the ability to visualize ablated lesions, and (3) aid in producing more accurate electrical maps. As a prototype for the development of new approaches to electrophysiologic testing and catheter ablation, this proposal addresses atrial fibrillation primarily. The imaging technologies developed in this project, should however, be broadly applicable to using MRI to guide interventional procedures in the heart in general, as well as in other organ systems.

STATUS OF RESEARCH AND PARTNERSHIP:
We have added a partner, Irvine Biomedical Incorporated, which is a manufacturer and supplier of electrophysiology catheters. They have produced MRI compatible electrode catheters per our specifications, that are suitable for use in humans. We have applied for an Investigation Device Exemption (IDE #G010093) for MR-guided electrophysiology studies and catheter ablation in patients. Safety data has been completed, showing that standard MRI pulse sequences do not cause unacceptable heating of the MRI-compatible electrode catheters (Investigator specified, custom manufactured by Irvine Biomedical and Bard Electrophysiology). We have received a response from the FDA, and the only significant issue is generating data indicating the electrical and mechanical integrity of the catheters. Irvine Biomedical has supplied the data, we are completing final safety studies of the human-grade catheters, and hope to be approved and start studies in a few months. We have also deleted a partner, Surgivision, as we found that the technology developed by them appears not be applicable to this project at this time.

We did additional patient studies pre and post ablation, as well as imaging normal controls for anatomic

comparisons. We did systematic studies of the heating produced by imaging the electrode catheters. For MRI scanners, the FDA sets limits on the allowable power deposition measured by peak specific absorption rate (SAR, 8 W/kg), and temperature change (2 degree C in torso). SAR was measured at the tip of an electrode catheter which was placed in a 10 L saline-filled phantom for 90 different geometric configurations to find the worst-case condition. In addition, temperature probes were attached to the tips of three electrode catheters, and the catheters were positioned in the hearts of 4 dogs. With standard imaging protocols, SAR and temperaure increases were within FDA limits.

We also demonstrated that MRI identifies the maximum gap in linear ablative lesions associated with conduction block. In 10 dogs, a linear lesion with a 3 cm gap was created over the distal right ventricle by dragging a saline-cooled RF probe over the epicardial surface. Conduction through the gap was assessed with a 3 x 4 array of bipolar electrodes (15 mm x 24 mm). The gap was gradually decreased by additional RF application until conduction through the gap was blocked (defined as the appearance of double potentials and a change in the conduction sequence). Using a high-resolution MRI coil, we visualized the ablated lesions and gap using a T2 weighted black blood, cardiac gated fast spin echo MRI sequence. After imaging, the hearts were removed, and histologic sections were taken through the lesions and gaps. Gap lengths measured by histology and MRI were highly correlated ($r2 = 0.85$, $p < 0.0001$). Thus we demonstrated that MRI appears useful in assessing the completeness of linear lesions.

We have also completed biophysical studies on the time course of lesion visualization, which show that lesions can be imaged a few minutes after RF is applied, and that the appearance does not change significantly for up to 12 hours. This is important for timing of imaging studies post ablation. We have done studies comparing the MRI appearance of lesions with histopathology, showing that different types of pathology have different appearances on MRI. These studies may lead to improved understanding of the specific effects of ablations in individual patients. We have also completed studies showing that at least 3 electrode catheters can be placed in appropriate positions in the heart using real-time MRI guidance. This latter study is critical to demonstrating the practicality of MR guided studies.

For the coming year, technological development will continue. We are nearly ready to test an improved, combined electrode catheter, receiver coil. We will continue development of the MRI tracking system. This system is being integrated into catheters and initial testing in the scanner should commence in the next year. Further enhancements will include using the data from the tracking system to dynamically manipulate the imaging plane to keep it in the plane of the catheter tip.

We have obtained a high-performance graphics workstation, and we plan to develop enhanced software to allow real-time or near-real time display of cardiac structures in 3-dimensions during interventions, with superimposed catheter-tip-localization and electrical maps. We have added two graphics programmers to facilitate this software development.

As soon as our IDE is approved by the FDA, we will commence MRI-guided electrophysiologic studies and catheter ablations in humans. We plan on starting with simple electrophysiologic studies where 3 catheters will be placed under MRI guidance and intracardiac electrograms will be measured and compared with those obtained under standard x-ray fluoroscopic guidance.

ISSUES:
We have added and deleted partners, and are pleased with the flexibility of the BRP, as these changes have enhanced the overall program. We would like to know what criteria would be used for BRP renewals.

PI: HASEGAWA, BRUCE, PH.D.
UCSF Physics Research Laboratory
389 Oyster Point Blvd., Suite 1
South San Francisco, CA 94080
T: (415) 502-4494
bruceh@itsa.ucsf.edu

PROJECT TITLE: Imaging Structure and Function in Small Animals

PARTNERS' NAMES AND AFFILIATIONS:
Photon Imaging, Inc (Northridge CA)
Jamco Engineering (Cottage Grove OR)
Genentech Inc (South San Francisco, CA)
Michael W. Dae, M.D., UCSF (San Francisco, CA)
Harrison Barrett, Ph.D., University of Arizona (Tucson, AZ)

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI), National Institute of Biomedical Imaging and Bioengineering (NIBIB)

ABSTRACT:
This bioengineering research partnership will develop a dual-modality CT/SPECT system for high-resolution imaging of radionuclides in transgenic and knockout mice that now are in widespread use to model the mechanism, diagnosis, and treatment of human diseases. This research will focus on the development of techniques that correlate structure and function, and that can perform noninvasive and quantitatively accurate measurement of tissue metabolism and organ physiology in small animals using radiolabeled tracers. The research program includes 5 specific aims. (1) A pinhole SPECT system will be developed for radionuclide imaging of small animals. Two interchangeable detector arrays will be developed, one for imaging low-energy radionuclides such as 125I (27.5 keV), and the other for imaging 99mTc (140 keV) and other radionuclides having higher photon energies. (2) The pinhole SPECT system will be integrated with a cone-beam computed tomography system volume to allow sequential acquisitions of CT and SPECT images without moving the animal. (3) Cone-beam tomographic algorithms will be implemented for reconstructing the radionuclide and x-ray tomographic data from the small animal imager. Techniques will be developed that use the reconstructed CT and SPECT data to quantify regional distribution of radionuclide concentration at spatial resolutions suitable for mice. (4) The dual-modality imaging system will be used for in vivo measurement of cardiovascular physiology in transgenic mice to investigate the role of the sympathetic innervation in heart disease. (5) The dual-modality imaging system will be used to measure the tumor and organ distribution of humanized anti-HER2 monoclonal antibody in a transgenic mouse model of metastatic breast cancer. The overall goal of this project will develop a high-resolution imaging system that combines CT and SPECT to correlating structure and function. The system also will be designed to perform noninvasive serial studies in mice, and to replace invasive direct tissue sampling and autoradiography for biodistribution studies and functional assessments using radiolabeled tracers in transgenic mice.

STATUS OF RESEARCH AND PARTNERSHIP:
Year 1 of the BRP effort is focused on developing conceptual designs of the proposed small animal imaging system, as a prelude to detailed design and eventual assembly and implementation. The primary subsystems under development include the CT subsystem (led by UCSF), the radionuclide detector and read-out subsystem (led by Photon Imaging Inc.), the imaging system gantry (led by Jamco Engineering), and system control electronics (led by UCSF). UCSF has held two design meetings with Photon Imaging, and is planning a third design meeting to include UCSF, Photon Imaging, and Jamco Engineering in April 2002. In addition, the BRP will initiate a quarterly seminar

series focused on the topic of small animal imaging. This will enable partnership members to keep abreast of developments in small animal imaging, and to allow potential users at UCSF, Genentech (partner organizations), and elsewhere have the opportunity to participate in and be aware of the status of the development effort.

ISSUES:

Years 1-3 of the BRP are directed primarily at engineering development of the proposed small animal imager. The primary effort during this initial period will be from the engineering partners, UCSF Physics Research Laboratory, Photon Imaging Incorporated, and Jamco Engineering. As the program proceeds, then we will increase the participation of Genentech Incorporated and UCSF collaborator, Michael Dae, M.D. Dr. Harrison Barrett at the University of Arizona will serve primarily in the role of a consultant and external reviewer of the project, and will be consulted to provide expertise and to review of the project. In addition, the UCSF Physics Research Laboratory (PRL) is receiving funding under a "Campus Laboratory Collaboration" (CLC) from the University of California Office of the President. The CLC involves the PRL, the x-ray astronomy group at Lawrence Livermore National Laboratory, and the Space Sciences Laboratory of the University of California, Berkeley, and is directed at the development of high-resolution gamma-ray detectors for small animal imaging and other imaging applications. The CLC will parallel but will not overlap the effort in the BRP. Our goal is to use the CLC to develop high-resolution gamma-ray detectors that could be implemented and tested on the imaging system being developed by the BRP, and in a way that would facilitate and enhance the design of future small animal imaging systems.

PI : **HIRSCHL, RONALD B.**
F3970 Mott Children's Hospital
Ann Arbor, MI 48109-0245

PROJECT TITLE: Total Liquid Ventilation: A Bioengineering Partnership

PARTNERS' NAMES AND AFFILIATIONS:
James Grotberg, Ph.D., M.D.
Biomedical Engineering Department
University of Michigan

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
ARDS is a frequently lethal pulmonary process that occurs in approximately 150,000 patients each year. Total liquid ventilation (TLV), in which the lungs are filled with perfluorocarbon and ventilated with a device which oxygenates and removes carbon dioxide from the perfluorocarbon, has great potential to effectively treat patients with ARDS. The clinical principal investigator has been performing studies in liquid ventilation over the last 8 years. Through our laboratory effort, we have generated data that demonstrate the efficacy of TLV in improving gas exchange, pulmonary function, and oxygen delivery, as well as in reducing acute lung injury. The bioengineering principal investigator has been performing studies in biofluid mechanics and transport of the pulmonary system for many years. This proposal addresses several fundamental physiological and bioengineering issues that underlie the progress toward establishing TLV as a clinical tool: 1) the optimal means for administering the liquid into the lungs; 2) the effect of ventilation parameters upon gas exchange; and 3) the expiratory flow limitation which restricts the effectiveness of the technique. The current research proposal is, therefore, directed at developing a new partnership between a clinician scientist and a bioengineer in the investigation of these issues which involve principles of fluid delivery and distribution, gas transport, and flow limitation during expiration. Specifically, our investigation will assess the distribution of the perfluorocarbon with regard to rate of fill, position during filling, and the characteristics of the perfluorocarbon. Secondly, we intend to investigate and to model the parameters which affect gas exchange during TLV, such as tidal volume, respiratory rate, and lung distension, and to model local flow patterns within the airways and alveoli. Finally, we plan to assess the relationship of flow limitation during expiration to the rate of flow and the state of inflation of the lungs and to investigate strategic means of manipulating parameters which determine flow limitation. A thorough understanding of these issues and solutions to these problems will be critical to the clinical application of this new and exciting technology.

STATUS OF RESEARCH AND PARTNERSHIP:
Our research in the above project has progressed well. We have developed data evaluating the effect of rate of perfluorocarbon administration upon the homogeneity of the distribution of perfluorocarbon in the lungs. We have also characterized the effect of perfluorocarbon flow rate and lung volume upon the development of flow limitation and have defined predictors of the onset of flow limitation which will allow servoregulation of liquid drainage from the lungs and avoidance of airway collapse. Finally, we have begun to assess the relationship of ventilatory parameters during TLV to gas exchange and the effects of bronchodilation upon airway resistance and development of flow limitation.

Issues: We hold conferences involving all members of the partnership, along with trainees, every 2 to 4 weeks. Ongoing research is discussed and data presented at these meetings with vital input

contributed from both the bioengineering and clinical investigators. These conferences, with the associated integration of expertise, have resulted in a broader approach to our research.

We have numerous trainees involved in the partnership including two M.D. research fellows, one medical student, one bioengineering Ph.D. research fellows, 1 bioengineering Ph.D. candidates, and 1 bioengineering masters/undergraduate student. These trainees have been critical to sustaining the cross-fertilization and integration that has been valuable to this partnership.

ISSUES:
We have no specific problems with the partnership.

PI: **HOFFMAN, ERIC A**
Deptartment of Radiology
University of Iowa, 200 Hawkins Drive
Iowa City, IA 52242
T: (319) 356-1381
eric-hoffman@uiowa.edu

PROJECT TITLE: Image and Model Based Analysis of Lung Disease

PARTNERS' NAMES AND AFFILIATIONS:

University of Iowa: Geoffrey McLEnnan, M.D., Ph.D., Brian Mullan, M.D., Joseph Reinhardt, Ph.D., Milan Sonka, Ph.D., Ge Wang, Ph.D., Alan Ros, M.D., Timothy Timmerman, M.D., Hiroko Kitaoka.
Johns Hopkins University: Brett Simon, M.D., Ph.D. Marquette Univ. Anne Clough, Ph.D., Chris Dawson, Ph.D.
Purdue University: Frank Rosenthal, Ph.D.
Mayo Clinic: Erik Ritman, M.D., Ph.D.
Philips Medical: Shalabh Chandra, M.S.

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
This proposal brings together a multi-disciplinary team of investigators to focus on improving lung imaging, developing and validating imaging and image analysis protocols for quantitation of anatomic and physiologic lung features, and to develop a model of the normal human lung based upon these new measures. The unique measures that will be made possible by this research will be integrated into a model of the normal human lung. This model will provide for an atlas of the lung, lobes sub lobar segments and airway and vascular branching structure of the lung and attached to each level of this structure will be the normal range of the CT-based measures of regional lung physiology including ventilation, perfusion, and specific compliance as well as quantitative anatomic features including regional tissue texture and airway and vascular geometry. This model of the normal human lung, developed for three decades of adult age, will provide the comparative basis for detecting and quantitating pre-clinical and clinical distribution of disease.
As the core of this partnership, we will establish and maintain a state-of-the-art CT research scanning center which will allow the partners of this proposal access to the tools needed to investigate the basic principals of CT imaging and to engineer the methodology necessary to extract unprecedented detail of regional lung structure and function.

STATUS OF RESEARCH AND PARTNERSHIP:

1) **Imaging**
   a) Establish a scanning facility (State of the art, high speed CT to be used as a tool for the assessment of both multi-slice and dynamic imaging strategies for the advancing field of X-ray CT) Status: A multi-articulated, computer sensed armhas beenadded to the scanner. The arm position is tied to the scanner coordinates which allows for the trans-thoracic sampling of lung tissue with mm accuracy and with visual guidance tied to the volumetric CT image. A fluoroscopic C-arm has been integrated into the scanner to allow for on-site catheterizations.
   b) Establish methods for physiologic control and monitoring subject during scanning to assure standardization of scanning protocols Status: A pneumotach-based respiratory gating system has allowed us to successfully build a dynamic(breathing) volumetric image of the normal human lung while scanning humans during quiet breathing. We have subsequently implemented an inductance plethysmography (Respitrace)system and have found this method to be considerably simpler and more stable. The integration of respiratory gating into scanning has eliminated the absolute need for a breath hold when acquiring volumetric images of the lung. Breathold is still used when there is a need for the highest spatial resolution. This imaging method is now allowing us to evaluate the normal dynamics of the human airway tree.
   c) Improve tomographic reconstruction methods for superior temporal and spatial resolution without any significant beam hardening and scattering effects. Status: A mathematical model of the normal human lung from bronchus to alveoli has been implement, a CT scanning simulator has been developed. The first problem will are addressing with this system is in regards to identifying solutions to eliminate intensity variations from slice to slice occurring when imaging in a "half-scan" configuration of the scanner. Until this is solved, we are using full scans for our image quantitation which means that our scan aperture is 500msec.
2) **Image segmentation**
   a) Lungs, Lobes, Sub-Lobar Segments: Status: Lung and lung lobe segmentation has been achieved and is integrated into our comprehensive lung image analysis software: PASS or Pulmonary Analysis Software Suite
   b) Airways (central and peripheral) Status: Airway centerlines and branch points have been successfully extracted.

c) Vascular Tree Status: Under Development

d) Tissue characterization Status: We are now in the process of re-training the tissue characterization software for the new scanner and as part of this process we are imaging normal human subjects in 3 decades of age range.

3) Image matching

a) Image Registration based upon the shape of the lung and lung lobes, airway and vascular branching structure. Status: lung and lobar registration implemented and has been refined to taking into account internal landmarks. An additional matching system has been implemented which uses airway tree branch points to link one image set to another. The system has been refined to allow recovery if there are inconsistencies between the two image data sets in regards to which branch points have been segmented.

4) Physiologic analysis

a) Ventilation: Characterize Xenon washout measures of regional ventilation and compare with measures of specific compliance in animals and humans Status: Xenon wash-in model has been expanded to provide not only regional ventilation but also an estimate of regional perfusion and thus an estimate of V/Q

b) Perfusion/Ventilation: In isolated and intact canine lungs and under various conditions of stress: Characterize the flow models used to calculate parenchymal perfusion from CT-based time intensity curves Status: Integrated, PC-based package implemented to deconvolve flow curves for assessment of micro vascular flow parameters along with ventilation parameters from the use of stable xenon. Regional Ventilation/Perfusion maps have been measured successfully via multi slice Philips Scanner. These V/Q maps using both xenon and injected iodinated contrast agent are now being compared with the xenon only method for assessment of V/Q. We have also established a collaboration with Amersham who manufacture the radiopaque non-ionic contrast agents. They have formulated an experimental agent which is expected to maintain a tighter bolus for a longer time duration which should allow us to inject the contrast at a more distal site than our current use of the right ventricular outflow tract. If successful this will help considerably for the transition of our methods to humans.

c) Evaluation of the utility of the physiological measures, along with tissue characterization from 2-d by following early progression of lung pathology in a dog model of emphysema. Status: sheep model of emphysema established and methods for tracking pulmonary function simultaneous to CT scanning have been implemented. We have established methods for measuring pulmonary function (FEV1, compliance, etc) in the sheep. These studies are now fully underway. We have also established a unique microscope facility which includes a computer controlled large field (12x18 cm) microtome and a wide angle of view surgical microscope. The fully integrated system, which includes a 12 mega pixel air cooled digital camera, is allowing us to fully reconstruct whole lung specimens with 4-40 micron resolution. The goal is to achieve precise alignment of the volumetric microscopic image of the lung with the in vivo characterized image data.

5) Construction of the lung model

a) Development of the data-structure linking lung structure to function in normal male and female humans Status: Data Structure for the establishment of the lung model has progressed and we have solved many of the logistics problems regarding how to deal with the fact that lung structure is similar but not identical across Individuals.

6) Application of the model to detection and quantification of pathologic processes

a) Correlation of in-vivo measures with ex-vivo measures of lung lobes surgically removed for solitary pulmonary nodules Status: not yet initiated.

b) Assessment of the early effects of smoking (through scanning of non-smokers and smokers with normal ranges of pulmonary function test parameters) Status: scanning under way.

There have been three new projects which have sought to join the partnership or to expand upon the individual role in the partnership. A supplement request was submitted by Michael Hlastalla out of the University of Washington in Seattle seeking to establish the multiple-inert gas elimination technique for comparison with CT based measures of regional pulmonary V/Q. Rick Albert of Denver General Hospital/Univ of Colorado Medical Center has submitted an R01 request to study ventilatory methods in ARDS using an sheep model, and Brett Simon of The Johns Hopkins University has submitted an R01 to evaluate a sheep model regarding mechanism of lung injury and the use of surfactant replacement methods. Numerous company interactions have derived from various aspects of our BRP including contrast agent development with Amersham and respiratory gating means in collaboration with Ultraguide and Philips. Software derived from this BRP has found use in several SCORs and is being used by the National Emphysema Treatment Trial (NETT) in which CT measures are being investigated as ourcomes predictors for lung volume reduction surgery.

ISSUES:

The supplier of clinical grade Xenon Gas, Prax Air, ceased to produce the gas. We have identified a new source for the gas, but there will be a delay for human use approval. As we increase our success, the ability to host the partners while at the same time focusing on the Iowa portion of the R&D has proven a challenge but we have put in place several solutions. One has been to cross train members of the laboratory to function in quite varied capacities.

PI: **HOLLISTER, SCOTT J.**
Room 3414 GG Brown
2520 Hayward
Ann Arbor, MI 48109-2125
T: (734) 647-9962
scottho@umich.edu

PROJECT TITLE: Engineering Joint Scaffolds for Concurrent Function and Regeneration

PARTNERS' NAMES AND AFFILIATIONS:
Dr. Robert Guldberg, Assistant Professor of Mechanical Engineering, Georgia Institute of Technology, member of Georgia Tech Tissue Engineering NSF Research Center
Dr. Kristi Anseth, Associate Professor of Chemical Engineering, The University of Colorado

Consultants:
Dr. Stephen Gordon, Vice President of Advanced Technology, Osiris Therapeutics, Inc.
Dr. Susan Herring, Professor of Orthodontics, University of Washington
Dr. Jeffrey Hollinger, Professor of Biomedical Engineering, Carnegie Mellon University, Director of Bone Tissue Engineering Center at CMU
Dr. Louis Mercuri, Professor of Oral and Maxillofacial Surgery, Loyal University Chicago
Dr. Stephen Milam, Professor and Chair of Oral and Maxillofacial Surgery, The University of Texas Health Science Center, San Antonio

GRANTING NIH INSTITUTE/CENTER: National Institute of Dental and Craniofacial Research (NIDCR)

ABSTRACT:
Tissue engineering offers considerable promise for temporomandibular (TMJ) joint reconstruction, a pressing clinical problem. To create durable engineered joint implants, the effects of scaffold material and architecture on tissue regeneration and function must be understood. To fill this vital need, we must be able to systematically study controlled scaffold architecture effects on bone regeneration, bone-cartilage regeneration, and load bearing capability. In this BRP, we will determine the effects of designed and fabricated internal architectures on bone regeneration by bone marrow stromal cells in an in vivo model of osteogenesis. We will mechanically test these architectures to determine load carrying capability. To test bone-cartilage interface regeneration in vivo, we will create a scaffold interface design seeded with bone marrow stromal cells on one half of the scaffold (bone side) and auricular chondrocytes on the other half (cartilage side), creating a bone-cartilage interface inside the scaffold. Finally, we will then engineer a prototype Conylar Ramus Unit (CRU) based on the most promising data from the bone-bone and bone-cartilage scaffold studies. The primary goals of this BRP are to:

1) Determine how two scaffold materials (hydroxyapatite (HA) and polyanhydride) and four porosity variations within controlled architectures affect bone regeneration and load carrying capability.
2) Determine how scaffold interface designs using HA and polyanhydride for the bone half and polyanhydride and PGA for the cartilage half affect bone-cartilage interface regeneration
3) Test one prototype CRU scaffold that incorporates the best results from 1 and 2 in an in vivo minipig model at 3, 6 and 12 months. The prototype CRU will have designed external shape and scaffold architecture.

Our first two specific aims are to apply image-based optimal design and solid free-form fabrication to create the scaffolds. The remaining four specific aims are to investigate the performance of these scaffolds mechanically and using subcutaneous models, resulting in the in vivo minipig test of a prototype CRU.

STATUS OF RESEARCH AND PARTNERSHIP:
There are six specific aims for our BRP:

1. Optimally design bone scaffold architecture topology to match bone stiffness as closely as possible while maintaining a fixed porosity. Design ellipsoid pore scaffold interfaces with two different pore sizes that mimic cartilage matrix architecture.
2. Fabricate the bone and bone-cartilage interface scaffold designs from hydroxyapatite, polyanhydride and polyglycolic acid.
3. Determine how optimally designed scaffolds with different scaffold pore size and material affect scaffold strength and degradation. Specifically, determine if designed scaffolds have ultimate strength greater than mandibular condyle stresses.
4. In bone scaffolds having the same controlled microstructural topology, determine scaffold material and porosity

effects on bone regeneration as a function of time in a subcutaneous model.

5. In bone cartilage interface scaffolds having the same controlled microstructural topology, determine scaffold material and porosity effects on bone-cartilage interface regeneration as a function of time in a subcutaneous model.
6. Combine the best bone scaffold design with the best bone-cartilage interface scaffold to engineer a prototype CRU for placement in an in vivo minipig model.

Since receiving our BRP award in September, 2001, we have finished work on specific aim 1 and made substantial progress on specific aim 2. For specific aim 1, we have shown that it is possible to optimally design scaffold architecture to match Minipig condyle bone stiffness values published by Dr. Susan Herring, a consultant on our BRP, while maintaining a fixed porosity. The results have been submitted for publication in Biomaterials. The graphs in Figure 1 show that we can design scaffold architecture such that both the scaffold itself and the subsequent ingrown tissue will match native bone stiffness.

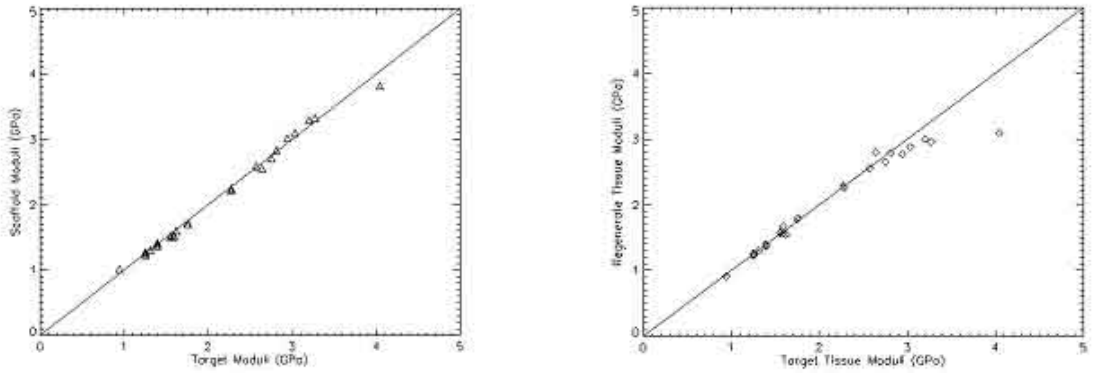


Figure 1. Comparison between (a) scaffold and native bone moduli and (b) regenerate tissue and native bone moduli based on optimization results.

For specific aim 2, we have developed a wide range of solid free-form fabrication based techniques to manufacture ceramic scaffolds, polymer scaffolds, and both discrete and blended ceramic-polymer composite scaffolds (Figure 2). We are preparing the manuscript for this work and will submit it shortly. This is critical for our mission of creating joint scaffolds since the cartilage portion of the joint will require a polymer scaffold while the bone portion may require a ceramic or ceramic-polymer composite scaffold.

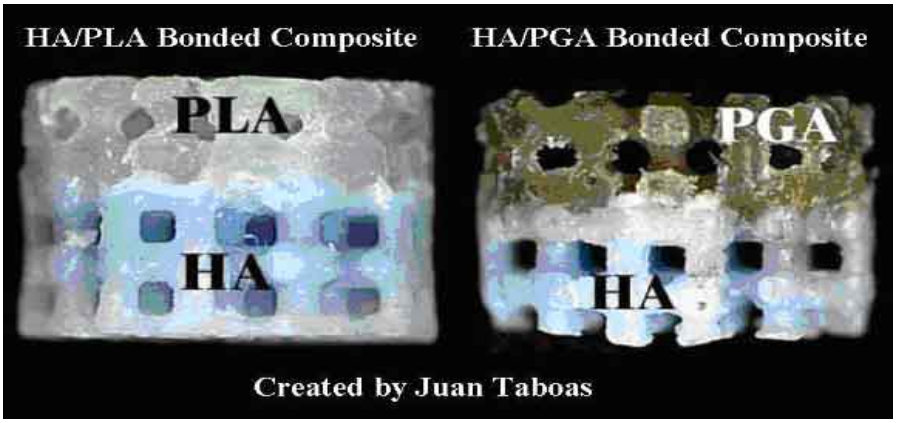


Figure 2. Example of (a) a composite HA/PLA scaffold and (b) a composite HA/PGA scaffold.

In addition to our accomplishments in research, we are developing a web site using a web based collaborative research tool from the University of Michigan. Both investigators and collaborators have access to the site which will be used to set up meetings, send announcements and track progress.

ISSUES:

The critical issues for this BRP will be 1) fabricating scaffolds for the bone portion of the scaffold that have sufficient strength and 2) generating both the cartilage and bone cartilage interface. For latter, we have proposed delivering chondrocytes, but we have also begun experiments using bone marrow stromal cells pulsed in vitro with TGF-b1 and insulin as a potential method of regenerating cartilage.

PI: **HUMPHREY, JAY D.**
Department of Biomedical Engineering
233 Zachry Engineering Center
Texas A&M University
College Station, TX 77843-3120
T: (979) 845-5558
F: (979) 845-4450
jhumphrey@tamu.edu

PROJECT TITLE: Histo-Mechanics & Biology of Remodeling in Hypertension

PARTNERS' NAMES AND AFFILIATIONS:
Texas A&M Dwight Look College of Engineering:
J.D. Humphrey, Ph.D., K.R. Rajagopal, Ph.D.
Texas A&M College of Veterinary Medicine:
T.F. Fossum, D.V.M., Ph.D., M. Miller, D.V.M., J. Stallone, Ph.D.
Texas A&M Health Science Center:
L. Kuo, Ph.D., E. Wilson, Ph.D., G. Davis, M.D., Ph.D.
Washington University, Department of Biomedical Engineering:
L.A. Taber, Ph.D.

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
Hypertension remains a major risk factor for a multitude of cardiovascular diseases, and as such it is responsible for significant morbidity and mortality. Recent advances in vascular biology and mechanics suggest a paradigm shift in hypertension research. It is now clear that focusing on local regulatory activities of the vascular wall that are controlled by mechanotransduction mechanisms promises significantly increased understanding. In this proposal, we will focus on the molecular mechanisms of vascular adaptation in coronary and cerebral arteries and arterioles, and the associated integrated manifestations in vessel morphology and function at the cellular and tissue levels. Toward this end, we have developed a new micro-pig model of renovascular hypertension that allows us to detail the time-course of hemodynamic changes during the development and reversal of the hypertension. Using an externally controllable suprarenal aortic coarctation model, we will delineate between purely mechanical effects and those due to engaging the renin-angiotensin system. This will allow us to explore the hypothesis that the efficacy of pharmacological therapy depends strongly on the target vascular bed and the time that the intervention is initiated during the development of the hypertension. The overall working hypothesis is that hypertension-induced alterations in cell function and matrix biology are largely due to changes in the point-wise multiaxial stress field. Specifically, we hypothesize that altered stresses (intramural and wall shear) induce (1) changes in the local expression of nitric oxide and angiotensin, (2) down-regulation of potassium-sensitive ATP channels and adenosine receptor subtypes, (3) increases in RGD integrin binding sites in the matrix, similar to those in a wound healing response, and (4) spatial and temporal differences in apoptosis and the production of growth factors and proteases. These effects, balanced by a resetting of the baroreceptor reflex, shear stress regulation of endothelial activity, and the myogenic response together result in the bed-specific adaptation. These hypotheses will be tested by combining clinical, molecular, cell biological, immunohistochemical, morphological, and biomechanical methods to study coronary and cerebral vessels (n = 5-8 per cohort) at multiple times during the development and reversal of hypertension in a single animal model - although there are many calls in the literature for multidisciplinary attacks on the problem of hypertension, this study will be the first to collect

and synthesize such broad data. Indeed, given the vast amount of data, we suggest that combining three recent, separate theoretical developments by members of our team will enable us to develop mathematical models that synthesize the data and provide predictive capability. The latter will enable the exploration of further hypotheses in an efficient manner and guide pharmacologic delivery strategies. Years 1-2 will focus on the time-course of changes due to the development of hypertension whereas years 3-5 will focus on the time-course of changes due to reversing the hypertension either mechanically or via specific pharmacological agents, both as a function of the time (during the development of hypertension) that the intervention is initiated.

There have been no changes in the partners or the organizational structure. As noted by the study section, the greatest promise and yet the greatest challenge in this research project is the attempt to synthesize data from such diverse sources (clinical, biomechanical, physiologic, cell biologic, histochemical, and molecular) into a mathematical model having both descriptive and predictive capability. Consequently, the first need was development of a general framework for the model based on observed changes in vascular structure and function due to hypertension. Toward this end, we have made significant progress, which is documented in two papers that have been accepted and a third paper that is in advanced preparation. Briefly, we have shown that one can exploit advantages of a full nonlinear continuum mixture theory that accounts for changing mass fractions, natural configurations, properties, and rates of turnover of the predominant structural constituents while avoiding potential complications thereof associated with the need to prescribe boundary conditions on (mathematical) partial tractions, etc. This is accomplished via a local homogenization of the stress response function that allows one to treat the vascular wall as a constrained mixture and thus ignore momentum exchanges between constituents. The constitutive structure further reveals those quantities (e.g., rates of constituent turnover) that need to be measured as a function of radial location in the wall and time of development. One engineering student has also made significant progress in developing a macro that partially automates the determination of spatial gradients in immunohistologically measured quantities.

ISSUES:

The primary "issue" early on resulted because each lab needs to perform tests on tissues harvested from each animal used in the study - hence we needed to get into place, and train, new graduate students (2 engineering, 1 medical pathology, 2 medical physiology, and 1 veterinary physiology) as well as 1 post-doc, 1 surgical resident, and 1 animal technician. Due to the timing of the start of the project (late September 2001) it has taken some time to get all the necessary people in place, trained, and coordinated. Training was/is being accomplished largely by having the new people assist in ongoing (related) experiments in the individual labs. Fortunately, in parallel we have been able to focus on the requisite theoretical advances, which define in large part the particular data sets that are needed once the penultimate experiments start (expected April-May 2002) for the 8 week hypertensive cohort.

PI: **HUSE, WILLIAM D., M.D., PH.D.**
Novasite Pharmaceuticals, Inc.
11095 Flintkote Ave.
San Diego, CA 92121
bhuse@novasite.com

PROJECT TITLE: Drug Discovery of Large-Scale Variant Targets by HTS

PARTNERS' NAMES AND AFFILIATIONS:
Juan Ballesteros, Ph.D., John T. Ransom, Ph.D., Larry A. Sklar, Ph.D.
Bruce Edwards, Ph.D., Eric Prossnitz, Ph.D.

GRANTING NIH INSTITUTE/CENTER: National Institute of Allergy and Infectious Diseases (NIAID)

ABSTRACT:
This project involves a collaboration between Novasite Pharmaceuticals, Inc. and the University of New Mexico (UNM). It involves development of a novel flow cytometry-based (FCM) high-throughput screening (HTS) system capable of screening and sorting thousands of cells bearing variant receptor targets simultaneously in real time. We will use this instrumentation to develop a novel approach to drug discovery via large scale generation and screening of variant targets, centered on identifying ligand-receptor interactions at large scale. The UNM team is developing the FCM hardware, while Novasite is utilizing proprietary technology to develop a library of receptor variants with a single base substitution per variant and a cell system where a single variant receptor type is expressed in a single cell. The combined advantage of the HTS FCM and variant target expression technology is a combinatorial explosion in the number of ligand-receptor interactions explored relative to one-receptor screening approaches. Our aim is an agonist for the cannabinoid-2 G protein coupled receptor (GPCR), which may prove useful as an anti-inflammatory and immunosuppressive agent. A general expression system efficiently tranfects one single variant target cDNA per cell in a single transfection step. We use a cell-based GPCR functional assay based on Ca2+ sensitive fluorescent dyes. The GPCR's binding site residues are randomized, resulting in thousands of receptor constructs with enhanced recognition properties, capable of recognizing novel and high affinity leads. For lead optimization, we will sort cells with lower EC50s than the wild type receptor. The variant GPCR present in these isolated cells represents a mutation that enhances the potency of a given lead. This data will be analyzed by computational molecular models, matching the variation of chemical moieties within the ligand with the variation of amino acid residues within the receptor to guide docking procedures. Translating the amino acid changes that enhance the lead's potency into mirror-image modifications proposed on the chemical compound will guide the lead optimization process.

STATUS OF RESEARCH AND PARTNERSHIP:
Novasite has focussed on acquiring flow cytometers (FCM) that will be suitable for modifications developed by the team at the University of New Mexico and on validating the utility of the systems for industrial screening. The instruments include a high-speed cell sorter (MoFlo, Cytomation Inc.) and an older FACSort instrument (Becton-Dickinson Inc.) with software and hardware upgrades for analyses without sorting. The modifications to the instruments are described in Dr. Sklar's summary as the HypercyteTM which enables sampling and test compound mixing at rates of at least 10 samples/min for analysis. The system is also being implemented on the high-speed cell sorter to enable high-speed sorting of those cells that interact favorably with test compounds at rapid compound introduction rates. This allows Novasite to achieve its goals of 1) screening for the ability of compounds to interact with single point-mutated variant receptors at the single cell level, and 2) isolate (sort) those cells bearing unique variant receptors that interact favorably with each

test compound so that its unique sequence can be identified. This allows us to build a precise understanding of the molecular, structural and chemical nature of the critical contact residues on the receptor involved in activation or antagonism. This will allow us to develop drug candidates with higher specificity, selectivity and affinity characteristics than previously possible. We have performed an initial screening characterization with the wild type 5HT2A receptor and commercially available serotonergic ligands. This initial test has validated the ability of FCM based single cell screening to identify the same hits as a more traditional cell-based screening system, a plate reader, which measures the average signal from thousands of cells. We have also validated the beneficial effects of screening mutant constructs by comparing hits identified with the wild type 5HT2A receptor with a single point mutant. The mutant receptor is recognizes new compounds representing different chemical classes. Furthermore, several compounds that are antagonists for the wild type receptor behave as agonists against the mutated receptor. This clear shift in compound activity indicates the critical nature of this residue in ligand recognition and consequent receptor activation. Data derived from mutant constructs, when analyzed in the context of a three-dimensional model of the receptor, enable us to identify the mode of interaction between these compounds and the receptor. Understanding how a drug candidate binds to the receptor will enable us to guide the lead optimization process resulting in more potent and selective drug candidates. This will validate the Novasite approach to drug discovery and lead optimization. Our final focus area has been to build an assay system so that we can develop an agonist for the CB2 subtype cannabinoid receptor, and to bring appropriate chemical synthesis and modeling expertise to aid the process. Towards that end we have recruited Dr. John Huffman (Clemson University) and Dr. Patricia Reggio (Kennessaw State University) as advisors to the project. Both have over five years experience with cannabinoid receptors. Dr. Huffman is supplying compounds with characterized affinities for both cannabinoid receptors and Dr. Reggio is providing modeling expertise. We have generated variant libraries of the wild type human CB2 receptor and are in the process of developing a suitable cell-based assay so that we may begin screening and optimization of the compounds using our unique variant receptor approach.

ISSUES:

An important issue has been a cell type specific sensitivity of the viable cells to shear forces in the system that can cause unexpected and confounding non receptor-mediated signaling events that can lead to a high background noise in the system. The UNM group is addressing the compatibility between the HypercytTM with the high-speed cell sorter we have in Novasite.

PI: **INTAGLIETTA, MARCOS**
Department of Bioengineering
University of California, San Diego
9500 Gilman Dr.
La Jolla, CA 92093-0412
T: (858) 534-4275
F: (858) 534-5722
mintagli@ucsd.edu

PROJECT TITLE: Bioengineering design of artificial blood

PARTNERS' NAMES AND AFFILIATIONS:

Prof. Vladimir Torchilin
Department of Pharmaceutical Sciences
Northeastern University, Boston, MA

Dr. Robert M. Winslow
President
Sangart Inc., La Jolla, CA

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

ABSTRACT:
Our objective is the design and development of artificial oxygen carrying plasma expanders (OCPEs) based on the modification of the hemoglobin molecule aimed at formulating an oxygen carrying fluid that has comparatively high viscosity, high affinity for oxygen, high oncotic pressure and that is economic in the use of hemoglobin, i.e., is effective with a minimal concentration of hemoglobin. These goals will be achieved via surface attachment polyethylene glycol (PEG) to the hemoglobin (Hb) molecule. Variables in PEG attachment include length and number of PEGs, bifurcations and bending moments. On biophysical considerations each variant has different solution properties, that may affect oxygen binding. A PEG formulation will be optimized in terms of cost, biological efficacy, COP, vasoactivity, vascular retention and viscosity. Physiological research in the microcirculation is used for further understanding the foundation of tissue oxygenation and is used to explore how alterations of blood physical properties affect tissue oxygenation and tissue survival in extreme hemodilution and shock. This program emphsizes the comprehension of the mechanism necessary for a stable balance between NO scavenging by molecular Hb in solution and the production of EDRF by shears stress dependant mechanisms. Different OCPEs will have different effects in this process leading to different types of vasoactivity. Our goal is to limit or eliminate vasoactivity while producing the most effective OCPE that restores or maintains microvascular function. In order to test the biological properties of OCPEs they have to be manufactured in a cost effective and practical manner. Bench top production will be used first and this will evolve into a self contained approach to be developed. Products will be characterized physically and biologically and readied for industrial production. Methods of production and purification will be optimized. Pre-clinical animals studies will be carried out to test methods and areas of application of developed products. Production and testing instrumentation will be developed to insure the effective use of developed products. Clinical trials will be designed.

STATUS OF RESEARCH AND PARTNERSHIP:
The focus of the initial activity has been the implementation of the research and development plan leading to the design of an effective product that can be manufactured and delivered at a cost that is competitive with blood. The problem of effectiveness was addressed by establishing a control baseline in terms of existing products. An array of microvascular tests were made at UCSD to determine the microvascular transport properties of an oxygen carrying bovine molecular hemoglobin solution manufactured by Biopure Inc. presently marketed for veterinary applications.

This product is similar to other products presently in various stages of clinical trails. Analysis of the effectiveness of this product was made by comparing how it affects functional properties of the microcirculation during extreme hemodilution and shock and comparing this with similar procedures carried out with conventional non oxygen carrying plasma expanders (dextran 70kDa, hydroxyethyl starch). It was found that this type of molecular hemoglobin based product provides no functional improvement over that attainable with conventional colloidal plasma expanders, supporting the need for a radically new approach which was attained by with MaleamidePEG-Hb. The efficacy of type of hemoglobin modification was tested for microvascular efficacy in the hamster window model, in hemorrhagic shock experiments in a rat model, and in a swine hemorrhage protocol carried out in collaboration with the Swedish Defense Establishment (FOI) in Stockholm, Sweden. Efficacy of this product was compared to treatment with hydroxyethyl starch (5 g/dl, Pentastarch, Brown Inc.). In general, and for the period of 2 hours following resuscitation, this product was found to be superior to conventional plasma expanders and blood in terms maintenance of functional capillary density, acid base balance, perfusion, and survival. This product has been submitted for evaluation in terms of toxicity, biodistribution, intravascular retention time and systemic cardiovascular effects in accordance to the requirements for application for clinical trials. Preliminary findings indicate that it passes all tests. Production facilities are presently being designed and implemented for Phase 1 clinical trials. Portable, self contained units for producing MalPeg-Hb from a donated unit of blood are in the design stage.

ISSUES:
No issues

PI: **IZATT, JOSEPH A.**
Duke University
Department of Biomedical Engineering
136 Hudson Hall
Durham, NC 27708
T: (919)660-5128
jizatt@duke.edu

PROJECT TITLE: Partnership for Research in Optical Coherence Tomography

PARTNERS' NAMES AND AFFILIATIONS:

Michael V. Sivak, Jr., M.D.	Case Western Reserve U., Div. of Gastroenterology (CWRU-GI)
Andrew M. Rollins, Ph.D.	Case Western Reserve University (CWRU-BME, CWRU-GI)
Calum MacAulay, Ph.D.	British Columbia Cancer Agency (BCCA)
Stephen Lam, M.D.	British Columbia Cancer Agency (BCCA)
Haisan Zeng, Ph.D.	British Columbia Cancer Agency (BCCA)
David M. Huang, M.D., Ph.D	Cleveland Clinic Foundation, Cole Eye Institute (CCF)
Hilel Lewis, M.D.	Cleveland Clinic Foundation, Cole Eye Institute (CCF)
Peter K. Kaiser, M.D.	Cleveland Clinic Foundation, Cole Eye Institute (CCF)
Raj Shekhar, Ph.D.	Cleveland Clinic Foundation, Biomedical Engineering (CCF)
Cynthia A. Toth, M.D.	Duke University, Duke Eye Center (DUEC)
Matthew R. Glucksberg, Ph.D.	Northwestern University, Biomedical Engineering Department (NU)
Jeffery W. Kiel, Ph.D.	U. Texas Health Science Ctr. San Antonio (Consultant to NU)

GRANTING NIH INSTITUTE/CENTERS: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

ABSTRACT:
This Biomedical Research Partnership proposal represents a multidisciplinary approach to advance the state of the art in diagnostic anatomical and functional imaging in situ at the micron scale. This will be achieved by developing fundamental advances in the technology of Optical Coherence Tomography, validating new techniques using animal models, and employing new technologies in pilot clinical studies. Optical Coherence Tomography (OCT) is a novel imaging technique based on infrared light reflectometry, which is capable of achieving micron-scale spatial resolution imaging non-invasively in human tissues. The initial clinical applications of OCT in ophthalmology have been successful, however, significant advances in OCT are now possible such that this nascent technology is on the threshold of finding applications with much wider clinical impact, particularly in minimally invasive early cancer detection. In addition, we will develop and apply novel technologies for structural and functional imaging in ophthalmic applications. Our Partnership includes biomedical engineers and clinicians from five institutions with demonstrated leadership in the transfer of optical diagnostic technologies to clinical practice. The **specific aims** of the proposal and the institutions involved in each (abbreviations defined above) are: **1.** To enhance and expand the clinical utility of Optical Coherence Tomography by developing the following core technologies: i) high frame rate imaging, ii) ultrahigh resolution imaging (<5 microns), iii) minimally invasive endoscopic and ophthalmic delivery systems, and iv) imaging of physiological function including blood flow and tissue hydration (CWRU-BME). **2.** To apply these technologies for pilot studies of early cancer detection in the gastrointestinal tract (CWRU-GI, CWRU-BME). **3.** To apply these technologies for studies of chemoprevention and early cancer detection in the lung (BCCA, CWRU-BME). **4.** To improve the accuracy and safety of keratorefractive surgery by developing OCT technology to measure the corneal epithelial remodeling response, structural stability, and hydration changes following laser in-situ keratomileusis (LASIK) (CCF, CWRU-BME). **5.** To improve imaging of retinal, sub-retinal, and vitreous pathologies with increased resolution and reduced motion artifacts (DU, CCF, CWRU-BME). **6.** To develop and test technologies for quantitative detection of blood flow in the retina and choroid of animals, and to apply these technologies to monitor patients with vascular complications of diabetes, glaucoma and retinal occlusive disease (NU, DU, CCF, CWRU-BME).

STATUS OF RESEARCH AND PARTNERSHIP:

Specific Aim #1: Develop core technologies for OCT With the move in July 2002 of the principal investigator to the Biomedical Engineering Department at Duke University (DUKE-BME), technology development is now progressing at both CWRU-BME and DUKE-BME partnership sites. Application-specific, video-correlated, real-time OCT imaging systems have now been deployed in clinical trials at CWRU and Partner institutions for imaging the anterior segment of the eye (using a new, slitlamp-mounted telecentric scanning geometry), skin (using a new dual OCT/video recording handheld probe), and the gastrointestinal tract (using 2.9 mm diameter, endoscope-compatible catheter probes). Initial evaluations of extensions to these systems for the first real-time OCT imaging in the retina and for bronchoscopic OCT have been conducted, and both technologies are undergoing development in preparation for clinical trials. The CWRU-BME group has demonstrated the first real-time color Doppler OCT system which uses sequential-scan processing for high-velocity resolution (<0.5 mm/sec) quantitative blood flow mapping in living vessels; this system has been demonstrated in an animal model and is being readied for studies of human retinal hemodynamics at Duke.

Specific Aim #2: Endoscopic OCT (EOCT) for early cancer detection in the gastrointestinal (GI) tract. An international, multi-center trial testing the efficacy of endoscopic OCT for detection of dysplasia in Barrett's esophagus is continuing. This trial is based on the real-time EOCT scanning engine and catheter probes developed by the CWRU-BME and CWRU-GI groups, in collaboration with Olympus Corporation.

Specific Aim #3: Bronchoscopic OCT (BOCT) for studies of chemoprevention and early cancer detection in the lung. Funding for this specific aim commenced in the second year of the project. The BCCA OCT system has been constructed and a probe for in vitro studies and excised tissue measurements is now functional. Design work on next-generation probe technology (<1mm diameter probe) is proceeding with evaluation of optical components. The development of instrumentation for confocal microendoscopy (work originally slated for the later years of the partnership) has continued and a 1.3mm diameter lens system fibre optic bundle probe has been constructed capable of ~1.4um x-y resolution and a ~10um axial resolution.

Specific Aim 4: Corneal OCT following LASIK. An arc-scanning OCT system was developed at CCF for corneal imaging. Image processing algorithms were developed to automatically measure the thickness profiles of the LASIK flap and cornea. The ability of OCT to measure corneal and flap thickness after LASIK has been demonstrated in a 6-month longitudinal study. Agreements with ultrasound measurements and laser setting were excellent. A new LASIK study has been initiated using the new telecentric (rectangular scanning) OCT system built in collaboration with CWRU-BME. The new system has sufficiently high speed (4000 lines/sec) to demonstrate the feasibility of 3-dimensional corneal topography/tomography. We have completed early studies involving over 50 patients in these new applications with very promising results.

Specific Aim 5: OCT imaging of retinal, sub-retinal, and vitreous pathologies. Dr. Toth and her colleagues have continued laboratory and clinical studies of OCT imaging of ocular tissues and macular disease and have new publications this year resulting from the NIH funding. The most notable finding this year, has been the finding that cystoid macular edema and retinal thickness, both identified by optical coherence tomography, correlate with vision loss in eyes with subfoveal neovascular membranes. The DUEC and DUKE-BME teams have completed the first protoype of the improved high-speed retinal OCT scanner, which is the first OCT retinal scanner to operate in near real-time (8 frames/sec). Initial images have been obtained in sample eyes; results will be presented at the Association for Research in Vision and Ophthalmology Meeting this year.

Specific Aim 6: Development of Doppler OCT for retinal and choroidal blood flow. The NU team has implemented a high velocity resolution, phase-resolved processing algorithm to investigate changes in choroidal perfusion. Experiments are being performed to ascertain whether phase shifts observed in the posterior eye resulting from red blood cell movement may be distinguished from cardiogenic motion. Efforts to improve contrast for Color Doppler OCT have lead to the development and use of high aspect-ratio silver nanoparticles as a vascular contrast agent. These specially designed plasmon resonance particles are extraordinarily efficient scatterers of a relatively narrow optical bandwidth (50-200nm depending on manufacturing processes), and preliminary results in phantom experiments show promise.
Issues: Communication between some Partnership sites is being augmented by reciprocal visits as needed, and particularly by frequent use of internet video conferencing between some sites.

PI: JACQUES, STEVEN L.
Oregon Health and Science Univerisity (OHSU), Dept. of Dermatology
Oregon Medical Laser Center, 9205 SW Barnes Rd., Portland, OR 97225
F: (503) 216-4092, -2422 FAX
sjacques@ece.ogi.edu

PROJECT TITLE: Biomedical Optics for Medical Research and Clinical Care

PARTNERS' NAMES AND AFFILIATIONS:
Dept. of Dermatology, School of Medicine, OHSU
Dept. of Electrical and Computer Engineering, School of Engineering and Science, OHSU
Oregon Medical Laser Center, Providence St. Vincent Medical Center

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

ABSTRACT:
This project develops the use of laser and light for medical research and clinical care. The uses include (1) measurement and diagnostic applications such as imaging and spectrosocpy, and (2) laser-tissue interactions such as laser micromachining and photodynamic therapy. **Aim 1:** Establish a Biomedical Optics Laboratory on the OHSU campus as a central resource supporting the interface of engineering centers with biomedical research and clinical studies**. Aim 2:** Initiate biomedical optics projects in the field of tissue engineering and biomaterials development. Current projects are (a) design of a reflectance-mode confocal microscope for imaging fluorescent cells in cartilage through the skin in mice genetically marked with green fluorescent protein (GFP), (b) design of optical speckle measurements of the microstrain of biomaterials and cells in response to mechanical stress. **Aim 3:** Initiate biomedical optics projects in the field of cancer detection, imaging and treatment, and cell biology. Current projects are (a) use of LIDAR techniques from atmospheric optics to imaging of cancer in superficial skin layers, (b) design of an optical fiber probe to recognize cancer, now being tested to distinguish oral melanoma from amalgam tattoos, (c) optically sensing the onset of mitochondrial swelling as a prelude to apoptosis elicited by photodynamic therapy (PDT), and practical application of PDT to the treatment of sarcoids in horses in collaboration with the Veterinary School at Oregon State University, (d) design of a spectral imaging camera to document changes in vasculature in cancer and in portwine stains and other vascular abnormalities. **Aim 4:** Develop the partnership between bioengineering and medical research by establishing a program identity which reinforces formal communication amongst participating investigators, staff, and students and represents a visible program to potential new collaboratores in the medical centers.

STATUS OF RESEARCH AND PARTNERSHIP:
Aim 1: A new Biomedical Optics Laboratory in now located on the OHSU campus near the animal facilities and the clinical care center. **Aim 2:** A reflectance-mode confocal spectrometer has been built and the degradation of confocal signal as a function of depth in light-scattering skin has been documented experimentally and modeled using Monte Carlo simulations of light transport. A microscope system for monitoring the movements of laser speckle spots within cells in response to applied stress has yielded first estimates of the Young's modulus for a cellular monolayer. **Aim 3:** A LIDAR system has been built that provides 60-um depth resolution and can be scanned to provide images of superficial skin. A spectral imaging system (CCD camera with tunable filter) and the associated analysis algorithm was built to enable images of skin cancers and portwine stain lesions and other vascular abnormalities that document the blood content, oxygenation status, and mean blood vessel depth, melanin content, and dermal scattering. This system has received approval for pilot clinical testing in Dermatology. **Aim 4:** The program now supports 5 graduate students and

involves 4 principal investigators with associated collaborators in other medical departments. An annual symposium is held at the Oregon Academy of Sciences. The OHSU Imaging Center has donated a confocal microscope to the program to build a system that incorporates advanced optical imaging techniques. In summary, the program has been established and is becoming a recognized resource in the OHSU community.

ISSUES:

This past year, the Oregon Graduate Institute of Science and Technology merged with the Oregon Health Sciences University to yield a new School of Engineering and Science with a newly named Oregon Health and Science University (OHSU). This merger has greatly enhanced the visibility of this BRP program on campus. On the down side, the transition from external grant subcontracts to internal grant accounts within one insitution has been suprisingly slow, but has now been established.

PI: **JAIN, RAKESH K.**
Massachusetts General Hospital and Harvard Medical School
Edwin L. Steele Laboratory, Department of Radiation Oncology
Massachusetts General Hospital
100 Blossom Street, COX 7
Boston, MA 02114
T: (617) 726-4083
F: (617) 726-4172
jain@steele.mgh.harvard.edu

PROJECT TITLE: Integrative Biology of Tumor Angiogenesis, Invasion and Metastasis

PARTNERS' NAMES AND AFFILIATIONS:

Project 1: Vascular Angiogenesis
Dai Fukumura, MGH
Donald G. Buerk, University of Pennsylvania
Paul L. Huang, MGH/Harvard

Project 2: Invasion
Yves Boucher, MGH
Michael Klagsbrun, Children's Hospital
Bruce Zetter, Children's Hospital

Project 3: Hematogenous Metastasis
Lance L. Munn, MGH
Josh Fidler, MD Anderson
Brian Seed, MGH/Harvard

Project 4: Lymphangiogenesis & Lymphatic Metastasis
Rakesh K. Jain, MGH
Kari Alitalo, Helsinki, Finland
Peter Carmeliet, Leuven, Belgium

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

ABSTRACT:
Now that numerous important genes associated with tumor angiogenesis, invasion and metastasis have been discovered, the grand challenge is to understand their function in **intact animals**. The second major challenge is to **integrate and apply** this knowledge to cancer prevention, detection and treatment. In the proposed BRPG, we will meet these challenges with a new, more precise, *quantitative, integrative and multi-disciplinary* bioengineering approach. This new bioengineering approach builds on unique and innovative techniques such as 1) genetically engineered mice to visualize gene expression, 2) *in vivo* models to visualize molecular and cellular events, 3) computer-assisted *in vivo* microscopy to quantify gene expression and function continuously *and* non-invasively at high (1-10mm) resolution in intact animals, 4) mathematical modeling to integrate the resulting information. Using this powerful technology, we will investigate four critical aspects of tumor metastasis: angiogenesis, invasion, hematogenous metastasis, and lymphangiogenesis & lymphatic metastasis. In the first year we will achieve several significant **milestones**: i) critically test the long-standing but unproven hypothesis that angiogenesis facilitates metastasis by increasing cell shedding; ii) establish a quantitative link between cell traction force and invasion through the tissue matrix; iii) demonstrate that stress generated by proliferating cancer cells can collapse lymphatic vessels in tumors; iv) provide the quantitative relationship between nitric oxide (NO) and angiogenesis in vivo. In the second and third years we will build on these findings to provide deeper quantitative insight into expression and function of three genes (NO synthase, VEGF-A, VEGF-C) considered critical to these four aspects of metastasis. Years four and five will see integration of these data in a unified framework and identification of strategies for clinical translation. The proposed BRPG offers a **new paradigm** for integrative studies of the dynamics of gene expression and function in cancer. With this new paradigm available to our collaborating partners working at the forefront of genomics and proteomics, this BRPG will facilitate **translation** of knowledge about the molecular biology of cancer into effective cancer prevention, detection and treatment strategies.

STATUS OF RESEARCH AND PARTNERSHIP: (July 1, 2000 - present)
We have made significant progress in all four partnership projects. The most significant development is the adaptation of a two-photon microscope for intravital microscopy critical to all four projects (Brown et al. 2002).

Project 1: Vascular Angiogenesis: In collaboration with Dr. Donald G. Buerk, we found that nitric oxide (NO) mediates angiogenesis in solid tumors and highly metastatic variant tumors produce more NO and

exhibit more but smaller tumor vessels. Chronic NO inhibition resulted in larger and less tortuous vessels. In collaboration with Dr. Paul L. Huang, we discovered that eNOS but not iNOS in host stromal cells contributes to angiogenesis and vessel morphogenesis in tumors. Although chronic NO inhibition lowers tumor tissue oxygen levels and slows initial tumor growth, response to vasoactive agents is increased suggesting improved tumor blood flow manipulability.

Project 2: Invasion: By using cellular and molecular reagents developed by Drs. Michael Klagsbrun and Bruce Zetter, we have shown that VEGF plays a dose-dependant role in cancer cell mobility. The degradation of collagen type I telopeptides (non-helical domain that participates in collagen polymerization) enhances tumor cell invasion.

Project 3: Hematogenous Metastasis: By using cellular and molecular reagents developed by Drs. Josh Fidler and Brian Seed, we have established an orthotopic model of renal cell carcinoma in mice that allows us to measure the rate of cell shedding by a renal tumor. The transcription profiles of these cells are currently being characterized using gene arrays.

Project 4: Lymphangiogenesis and Lymphatic Metastasis: In collaboration with Dr. Kari Alitalo, we discovered that intratumor lymphatics do not function despite the presence of the lymphangiogenic molecule VEGF-C and its receptors VEGFR2 and R3 in tumors (Leu et al, Cancer Research, 2000). Furthermore, in collaboration with Dr. Peter Carmeliet, we showed that VEGF-C increases angiogenesis and growth in tumors without altering leukocyte-endothelial interaction (Kadambi et al, Cancer Research, 2001). In collaboration with Dr. David Jackson (Oxford) and Dr. Tomarev (NIH), we showed that the recently discovered lymphatic marker (LYVE-1) is also present in the sinusoidal blood vessels of the liver, but absent in the primary and secondary liver tumors (Carreira et al., Cancer Research, 2001). Finally, we used two-photon microscopy to image deeper functional lymphatics (Padera et al., Molecular Imaging, 2002) and found them to be absent in both animal and human tumors (Padera et al., submitted). We also demonstrated that these tumors still metastasize to lymph nodes, in spite of the lack of functional intratumoral lymphatics, suggesting that lymphatics in the tumor margin are the pathway for lymphatic metastasis (Padera et al., submitted).

Publications

Fukumura et al., Predominant Role of Endothelial Nitric Oxide Synthase in VEGF-induced Angiogenesis and Vascular Permeability, PNAS, USA 98:2604-2609 (2001).

Leu et al., Absence of Functional Lymphatics within a Murine Sarcoma: a Molecular and Functional Evaluation, Cancer Research, 60: 4324-4327 (2000).

Kadambi et al. Vascular endothelial Growth Factor (VEGF)-C Differentially Affects Tumor Vascular Function and Leukocyte Recruitment: Role of VEGF-Receptor 2 and Host VEGF-A, Cancer Research 61:2404-08 (2001).

Mouta Carreira et al. LYVE-1 is not restricted to the lymphatic vessels: Expression in normal liver blood sinusoids and down regulation in human liver cancer and cirrhosis. Cancer Research 61:8079-84 (2001).

Brown, E. B. et al. In vivo measurement of gene expression, angiogenesis and physiological function in tumors using multiphoton laser scanning microscopy. Nature Medicine 7:864-8 (2001).

Padera et al. High-speed intravital multiphoton scanning laser microscopy of microvasculature, lymphatics, and leukocyte-endothelial interactions. Molecular Imaging 1:9-15 (2002).

Jain & Fenton. Intra-tumor lymphatic vessels: a case of mistaken identity or malfunction. Journal of the National Cancer Institute (in press).

Monsky et al. Role of host microenvironment in angiogenesis and microvascular functions in human breast cancer xenografts: Mammary fat pad vs. cranial tumors. Clin. Cancer Res., (in press)

Izumi et al. Herceptin acts as an anti-angiogenic cocktail. Nature, (in press)

Jain, Munn & Fukumura. Transparent window models and intravital microscopy: Imaging gene expression, physiological function and drug delivery in tumors. In: Teicher BA. Editor. Tumor models in cancer Research, pp647-671, 2001

ISSUES:

The Bioengineering Research Partnership is an ideal and innovative program to integrate bioengineering with molecular biology and molecular medicine, and to facilitate translation of new knowledge from genomics and proteomics to improving health care and quality of life.

PI: **KARELLAS, ANDREW, PH.D.**
Room S2-835, Department of Radiology
University of Massachusetts Medical School
55 Lake Avenue North
Worcester, MA 01602.
T: (508) 856 2069
andrew.karellas@umassmed.edu

PROJECT TITLE: Digital Mammography with a High Resolution Flat Panel Imager

PARTNERS' NAMES AND AFFILIATIONS:
Fairchild Imaging (formerly, Lockheed Martin Fairchild Systems)
1801 McCarthy Boulevard
Milpitas, CA 95035.

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI).

ABSTRACT:
This bioengineering research partnership between University of Massachusetts Medical School and Fairchild Imaging (formerly, Lockheed Martin Fairchild Systems) is aimed at developing and evaluating a new high-resolution flat-panel mammographic imager with variable pixel size (40-mm and 80-mm). The proposed next generation imager is a 2 x 3 array of large-area CCDs (8 cm x 8 cm) tiled in a seamless fashion to provide an imaging area of 16 cm x 24 cm. The CCD array is coupled to a structured CsI:Tl scintillator using non-tapering (1:1), straight fiberoptics, thereby preserving the spatial resolution without the detrimental loss in the collected signal, which is common with the older generation that use tapered fiberoptics. Our experience with the 100-mm pixel GE clinical evaluation prototype in a screening population appears to demonstrate equivalency for cancer detection with similar sensitivities. However, there are concerns about the ability to detect more subtle forms of calcifications such as punctate and amorphous. When calcifications are seen the edges do not appear to be as sharp as that observed with spot film views, which may be related to the relatively large pixel size (100-mm) of the detector. Hence, this investigation was undertaken with the specific hypotheses that: (a) The new imager will exhibit better detective quantum efficiency (DQE) than current screen-film technology. (b) Unlike current screen-film, the system will exhibit higher dynamic range. (c) The spatial resolution will be better than current flat-panel imaging systems due to the smaller pixel size. (d) The contrast will be significantly better than existing screen-film systems resulting in better visualization of breast anatomy at a reduced radiation dose to the patient due to the improved DQE. (e) A well-designed mammographic system driven in an optimized acquisition mode will replace screen-film systems for full-breast mammographic imaging. The research plan calls for preliminary computational and experimental studies followed by development and comprehensive evaluation of the system through objective and universally accepted metrics such as the spatial frequency dependent modulation transfer function and the detective quantum efficiency.

STATUS OF RESEARCH AND PARTNERSHIP:
The program began on July 1, 2001. Within the first seven months from the start of the program the following research activities have been accomplished:

1. Model development: A cascaded linear systems based model was developed to theoretically investigate the potential imaging characteristics of the proposed system. The model reiterates the results of our preliminary computations and provides support for the stated specific hypotheses.

2. Scintillator evaluation: Structured CsI:Tl scintillators from two possible vendors varying in thickness and manufacturing processes such as substrate, coating type and strength, were evaluated using a laboratory 1 inch x 1 inch back-illuminated CCD operating in the 24-mm pixel mode in terms of signal intensity, sensitivity, spatial resolution properties in terms of the modulation transfer function (MTF), and DQE.
3. Effect of binning: The effect of binning (grouping of pixels prior to readout) was studied in terms of the signal intensity, sensitivity, MTF and DQE using the laboratory 1 inch x 1 inch CCD.
4. Fiberoptic evaluation: Fiberoptic plates varying in material composition were evaluated for their x-ray attenuating properties. This study allowed optimization of the fiberoptic plate thickness to provide adequate shielding to the CCD from direct x-ray photon interaction. Currently, a new type of improved fiberoptic plate with increased core/clad ratio is being investigated in terms of its optical and x-ray attenuation characteristics.
5. Electrical properties of a single module: The electrical noise properties of a single module were investigated with the module operating at the maximum possible readout rate to evaluate the read noise and dark current characteristic at room temperature.

Indications from all the experimental and theoretical work completed so far indicate that this new generation of digital mammography system would provide the highest spatial resolution and improved DQE than the systems currently in clinical use. The partnership has been successful and the research has been progressing as per schedule.

ISSUES:
We have been conducting regular telephone conferences to exchange information with our bioengineering research partnership institution, Fairchild Imaging. There are no outstanding issues.

PI: **KOLLER, MANFRED R.**
Oncosis
6199 Cornerstone Ct., Suite 111
San Diego, CA 92121
T: (858) 450-7063
F: (858) 550-1774
fkoller@oncosis.com

PROJECT TITLE: Laser cell processing for basic and clinical research

PARTNERS' NAMES AND AFFILIATIONS:

Carlos Bachier, M.D.
Texas Transplant Institute (San Antonio, TX)

Esmail D. Zanjani, Ph.D.
VA Medical Center (Reno, NV)

Helen Heslop, M.D.
Baylor College of Medicine (Houston, TX)

James Leary, Ph.D.
University of Texas - Medical Branch (Galveston, TX)

GRANTING NIH INSTITUTE/CENTER: National Center for Research Resources (NCRR)

ABSTRACT:
PhotosisÔ is a technology platform that incorporates high-speed optical scanning of biological samples, image analysis, and computer-controlled laser-irradiation of specific targets within the sample for the purpose of inducing a biological response. Specific cells to be treated within a mixed population are identified by parameters such as size, shape, fluorescence, or other distinguishing features. Once identified, individual cells are targeted with a laser to induce a desired response, such as cell death, optoporation (for gene transfer), or even inactivation of a specific mRNA transcript within the cell. The current b1-prototype system can process hundreds of millions of cells in an hour under sterile conditions, making it useful for several research and clinical applications. In fact, this prototype has several advantages over other methods of cell processing such as flow cytometry, and this conclusion is supported by preliminary data shown within. Photosis has many potential uses, and this proposal brings together a number of institutions and researchers to investigate and define the possible applications of this novel technology. In its current configuration, the instrument uses a single color for cell detection and a laser to induce necrosis in every targeted cell. These capabilities enable the first application which is tumor cell purging from autologous NHL stem cell transplants, because such contaminating tumor cells are known to contribute to disease relapse. Additional applications will be developed, some of which will require modifications to the system design and building of new prototypes. The prototypes will be placed at four partnership sites where the basic and clinical applications research will be carried out, including: (1) in vivo study of purified stem cell subpopulations in the xenogeneic fetal sheep transplant model; (2) human clinical trials to assess NHL purging in autologous stem cell transplantation; (3) purification of genetically-modified stem cells and T-cells expressing a selectable transgene, as well as selective transduction of specific cells in a mixture via optoporation; and (4) accurate mRNA expression profiling from HIV-infected human stem cell populations. The proposed work will result in several types of novel bioengineering instrumentation for advancing the start-of-the-art in cell processing. These instruments will be used

in this program to advance basic and clinical research in stem cell biology, cancer, immunology, and genomics. Once developed, the resulting technology will be useful in other areas as well, some of which are described within.

STATUS OF RESEARCH AND PARTNERSHIP:
The clinical instrument for the tumor purging application has been completed, and is now undergoing pre-clinical validation in support of an Investigational Device Exemption (IDE) application to be filed with FDA. The clinical partner site (Dr. Bachier) will be engaged shortly to carry out the pilot clinical trial. A more flexible version of the Photosis platform to allow various types of non-clinical research is nearing completion. This instrument has benefited from the tumor purging instrument design, but with some critical additions: multi-color excitation and detection, multi-wavelength laser processing, and a more flexible user interface to facilitate research applications. Dr. Leary's will be the first site to receive the research instrument, as he has extensive experience in dealing with new cell processing devices. UTMB has been subcontracted for this research involving HIV-infected cells. As the follow-on research instruments are built, the additional sites will be installed and research will commence in those additional areas described above.

ISSUES:
FDA has requested additional data on the monoclonal antibody manufacturing and validation, delaying the initiation of the pilot clinical trial. These studies are underway, and should be complete by mid-year allowing the pilot trial to begin.

PI: **LANGER, ROBERT**
Massachusetts Institute of Technology
Department of Chemical Engineering
77 Massachusetts Ave, RM E25-342
Cambridge, MA 02139
T: 617-253-3107
rlanger@mit.edu

PROJECT TITLE: Microchip Drug Delivery System

PARTNERS NAMES AND AFFILIATION:
James Anderson, M.D., Ph.D.
Case Western Reserve University

Henry Brem, M.D.
Johns Hopkins Medical School

Michael J. Cima, Ph.D.
Massachusetts Institute of Technology

Venkatram Prasad Shastri, Ph.D.
Massachusetts Institute of Technology

GRANTING NIH INSTITUTE/CENTER: National Institute of Allergy and Infectious Diseases (NIAID)

ABSTRACT:
It is well known that the method by which a drug is delivered can have a significant effect on the drug's therapeutic efficacy. Most drugs have a concentration range in which they have maximum efficacy. Conventional drug delivery regimens result in sharp changes in systemic drug levels that can be toxic. Controlled drug delivery can alleviate the problems associated with conventional therapy by providing stable drug bioavailability in a therapeutically meaningful range and in addition can be used to localize the therapy to the tissue site of interest. We have shown that it is possible to fabricate a solid state silicon microchip in which a number of chemicals or drugs can be stored in individual micro-reservoirs and released on demand by electrochemically dissolving the gold cap in saline solutions with an external trigger. One advantage of this novel controlled release system is that it allows for simultaneous release of multiple drugs in complex release profiles. One can potentially develop a device that can be pre-programmed to deliver combinations of drugs in a pre-determined fashion. We believe that this novel delivery technology has broad utility in the biomedical areas such as local delivery of anesthetics for pain management, subdermal delivery of vaccines, periodontal delivery of antibiotic and anti-inflammatory agents, localized delivery of anti-tumor and neoplastic agents, gene delivery, delivery of antiarrhythmic agents. Based on the abovementioned rationale and our preliminary results we propose the following specific aims: (1) Development of an active, silicon based microchips for controlled release of drugs that can operate autonomously based on the electrochemical dissolution of a membrane over a drug containing reservoir, (2) Development of a passive, polymeric chip for the controlled release of drugs based on biodegradation of a polymeric membrane over a drug containing reservoir, (3) Evaluate the biocompatibility of active and passive microchip delivery device and (4) Evaluate the drug release both in vitro and in vivo, specifically: (a) show that predictable drug release is possible from both active and passive microchips (b) study a pathology such a s brain tumors that may be better treated by combination therapy (c) evaluate the efficacy of these devices in the tumor model.

STATUS OF RESEARCH AND PARTNERSHIP:
Advances were made in the operation of the micro-reservoir drug delivery devices during the past year. Silicon devices were used in vivo for the delivery of both fluorescein dye and radiolabeled BCNU chemotherapeutic agent in a subcutaneous rat model. The voltammetry profile was found to be critical for efficient in vivo gold removal and drug release. Both a cathodic cleaning cycle and sufficiently anodic voltage are necessary.

Fluorescein dye was released from several devices so that the spatial profile of the release could be assayed. The temporal profile of multiple releases from chips of radiolabeled BCNU was determined by plasma content. These studies are the first validation of the project concept in vivo and the first delivery of drug by MEMS technology in vivo. We are beginning the first in vivo release studies from the passive microchip devices in the next few weeks. Initial studies will release radiolabeled dextran, with subsequent studies investigating the release profiles of IL2 and BCNU.

The biocompatibility of the electrochemical dissolution of gold was tested using the cage implant system, in which samples of the material tested are placed inside wire mesh cages and the fluid inside is sampled periodically for cell counts and types. Devices were prepared so that the dissolution of a macroscopic piece of gold film could be performed inside a cage and the resulting cellular response monitored. Final results indicated that the main cellular response is due to the application of the voltage and creation of new surface area, and resolves over 3 days. A study in which the long-term reliability and biofouling of macroscopic gold electrodes implanted subcutaneously without cages was also performed. No changes in gold electrochemistry or platinum reference electrode behavior were seen out to 7 weeks. A study to evaluate the biocompatibility of three representative materials (PLA, PGA, and PLGA 10:90) is currently underway.

In vitro electrochemical tests were performed to compare gold corrosion behavior in serum and saline; similar thermodynamics but dissimilar kinetics were observed. The kinetic limitations of gold corrosion in serum were attributed to the protein adsorption onto the gold surface. This modification can be removed by cyclic voltammetry, which leads to a cycle to cycle current density growth in serum. Pitting corrosion was found to be an effective method of localized gold corrosion while releasing less gold chloride into the system.

A bulge test apparatus was constructed to pressurize the gold membranes and measure deflection using interferometry. Preliminary results show that the gold membranes can elastically deform at pressures as high as 60 psi. Currently we are studying the mechanical behavior of gold membranes corroded to different degrees to understand the membrane disintegration process. The bulge test also provides a reliable non-destructive method to evaluate the bottom nitride etching step in the microfabrication process, which is difficult to test using conventional methods.

Progress has been made in the development of a system to directly monitor the release of drug from microchip reservoirs. The technique measures the impedance of the reservoir contents as drug is released; the change in impedance is related to the rate of removal of drug from the reservoir. The impedance method was tested using a scale model of the drug delivery reservoirs. A special shadow masking technique was used to pattern the electrodes on the microfabricated reservoir walls. The next step will be to test the release-monitoring system using the modified drug delivery microchips.

The passive microchip devices have been used to release four pulses of a single chemical and two pulses of each of two chemicals from single devices. Near-infrared analysis of reservoir membranes showed no mixing of the membrane and substrate polymers in the membrane, supporting the premise that the substrate polymer does not affect the membrane degradation and chemical release. Current work focuses on three areas: 1) studying the membranes during release to understand the mechanisms by which they open, 2) analyzing the kinetics of chemical release to determine if there is a diffusion component, and 3) measuring the biological activity of heparin that has been released from devices.

ISSUES:

Currently the microchips studied in vivo are activated with external circuitry and power source necessitating the use of electrical leads coming from the device through the animal's skin to the ex vivo electronics. This need for transdermal leads causes problems with wound response as well as limiting the animal's potential mobility. An integrated circuit and on-board power source will be designed and tested. Packaging this circuit with the microchip will enable a small, single component device to be surgically implanted and control the potential waveform application for long-term release studies.

PI: **LEVINE, SIMON P. , PH.D.**
1C335 University Hospital
1500 E. Medical Center Drive
Ann Arbor, MI 48108-0032
T: (734) 936-7170
silevine@umich.edu

PROJECT TITLE: Direct Brain Interface Based on Event Detection in ECoG

PARTNERS' NAMES AND AFFILIATIONS:

The University of Michigan Direct Brain Interface research partnership is a collaboration which includes the Departments of Biomedical Engineering, Electrical Engineering and Computer Science, Physical Medicine and Rehabilitation, Neurology, Surgery and Radiology from the University of Michigan; the Department of Neurology from Henry Ford Hospital, and the Institute of Biomedical Engineering from the Technical University Graz.

GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke (NINDS)

ABSTRACT:
A number of people with physical disabilities have difficulty performing any physical movement and would benefit from a direct brain interface, an interface that accepts commands directly from the brain. Our BRP partnership addresses the functional evaluation of a direct brain interface (DBI) and the improvement of detection methods used to recognize specific brain activity.
The University of Michigan (UM) DBI system, which in part forms the basis for this research, uses a detection method based on time-domain template matching methods. Previous work with the UM-DBI has demonstrated good accuracy in off-line experiments. Current efforts are focused towards on-line implementation and testing with subjects at the University of Michigan and Henry Ford Hospitals who have implanted electrodes for purposes related to epilepsy surgery. (While these subjects are not members of the target user population, the presence of implanted cortical electrodes in these subjects provides a unique opportunity for direct brain interface development). The proposed functional evaluation includes: 1) Development of an on-line, real-time testing system for direct brain interface methods; 2) Examination of the ability of subjects to learn to voluntarily improve event-related potential (ERP) quality and detection performance given appropriate feedback; 3) Determination of the accuracy and speed with which a direct brain interface can be used to perform functional tasks; and 4) Identification of the relationship between the location of electrocorticogram (ECoG) recorded brain events and the activated portion of the brain as observed through functional magnetic resonance imaging.

Improvements in the accuracy by which brain events can be detected will be approached through evaluation and improvement of both time-domain (performed primarily at UM) and frequency-domain (performed primarily at Graz) ECoG based detection methods. In addition, off-line analysis will be used to 1) Investigate the ability of current detection methods to differentiate between brain activity related to different actions and 2) Determine the increased accuracy of event detection achievable using ECoG versus EEG.

The goal of this research is to demonstrate and evaluate the current and potential effectiveness of a direct brain interface for control of functional tasks based on the detection of human ERPs recorded from subdural electrodes on the surface of the brain. It is intended that DBI methods developed through this effort will also form the foundation for future generations of direct brain interfaces of ever increasing bandwidth and effectiveness. Beyond the scope of the proposed work, the results of these studies will form the foundation for clinical testing of the direct brain interface with individuals from target user populations using subdural electrodes.

STATUS OF RESEARCH AND PARTNERSHIP:

University of Michigan: On-line direct brain interface testing and time-based detection

Development of an on-line direct brain interface test system and on-line experiments with the system are underway. The EEG system that will form the basis of the system have been acquired. Software modules for the newly acquired EEG systems for this research are being developed and are in preliminary use. Development of the on-line DBI test system will continue and additional software modules (especially those for on-line feedback experiments) will be put into use as they become available.

The development and evaluation of improved single-channel detection methods is also underway. Both template based methods and template free methods are being examined. Initial results show that simple weighting of the template matching process based on the standard deviation of the signal did not improve detection. However, the Hjorth parameters of activity, mobility, and complexity have been shown to contain information that is useful for improved detection in combination with cross-correlation.

Graz Subcontract: Frequency-Based Algorithms

Adapting the Graz-BCI electroencephalogram (EEG)-based detection methods to accept ECoG was completed. This system implements the feature extraction methods 1) Band Power (BP), 2) Adaptive Autoregressive Parameters (AAR), and 3) Common Spatial Patterns (CSP).

The comparison of the UM-DBI cross-correlation based detection method with several Graz-BCI detection methods is underway. The Graz-BCI is based on event-related changes in oscillatory brain activity called event-related desynchronization and event-related synchronization (ERD/ERS), which can be visualized through ERD/ERS maps. The ERD/ERS visualization software was adapted to accept ECoG data. The maps were topographically arranged and labeled with the corresponding detection accuracy from current UM-DBI detection methods.

Results to date show that ERD/ERS activity can be found in almost all channels that also give good detection with the UM-DBI methods. Classification results using the Graz-BCI detection methods were calculated on an initial subset of the datasets using the BP (band power) and AAR (adaptive AR parameters) feature extraction algorithms. The resulting detection accuracies were similar to those achieved by the cross-correlation based method, although generally a bit worse. However, there are channels that show poor UM-DBI detection, but strong ERD/ERS activity. This gives rise to the hypothesis that ERP based methods and ERD/ERS based methods could possibly be combined to form a more robust and better performing DBI system.

Henry Ford Hospital Subcontract: On-line experiments

On-line testing of subjects at Henry Ford Hospital has not started yet since the equipment for such experiments has only recently become available. However, notification of the availability of subjects at Henry Ford Hospital is in place and arrangements are being made to deliver the EEG system for use at Henry Ford Hospital and perform the necessary safety tests.

ISSUES:

All collaborations are working very well. Recent implementation of web based work tools for discussions, live chat, and data exchange has further enhanced communications. The official start date for this grant was 4/1/01. However, due to issues related to equipment ordering and student recruitment, we have moved our effective start date forward to 9/1/01 and plan on continuing with this annual time frame for the duration of the grant.

PI: **LEY, KLAUS**
Professor
University of Virginia
Director, Cardiovascular Research Center
MR5 Bldg. - Rm. 1013
P.O. Box 801394
Charlottesville, VA 22908-1394
T: (434) 243 9966
F: (434) 924 2828
klausley@virginia.edu

PROJECT TITLE: Biomechanics of Leukocyte Adhesion Molecules

PARTNERS' NAMES AND AFFILIATIONS:

Michael Lawrence, Ph.D.
University of Virginia

William Guilford, Ph.D.
University of Virginia

Geoffrey S. Kansas, Ph.D.
Northwestern University Medical School

Jonathan Lindner, M.D.
University of Virginia

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
This BRP was initiated to conduct interdisciplinary bioengineering research in the area of molecular biomechanics. Leukocyte and endothelial adhesion molecules govern the trafficking of cells in inflammation, immunity, cancer metastasis and other processes. Some adhesion molecules, among them the selectins, are specialized to mediate adhesion in the presence of blood flow. Pressure-driven blood flow is associated with a shear stress exerted on the vessel wall, which results in a force on leukocytes and other cells trying to adhere to the endothelium. It is believed that adhesion under shear stress requires adhesion molecules with rapid association rates (on-rates), resulting in rapid formation of bonds. In vitro experiments and modeling studies indicate that the selectins also have high rates of bond dissociation (off-rates). Preliminary data suggest that the off-rates of selectins vary systematically with the shearing force exerted on the cell bound by the selectin (reactive compliance or tensile strength). In addition, the release of at least one of the selectins is accelerated by proteolytic cleavage by a surface-bound or membrane integral metalloproteinase. The BRP has four specific aims. (1) To measure the bond lifetimes and apparent off-rates of L-, P- and E-selectin bound to their natural ligands. (2) To determine the role of L-selectin shedding in regulating leukocyte adhesion and selectin kinetics. (3) To determine the impact of the selectin length and their cytoplasmic tail for the biomechanics of adhesion under shear flow. (4) To design and build beads, liposomes and gas-filled bubbles (ultrasound contrast agents) that use leukocyte adhesion molecules to bind to vessel walls under shear stress. Each of these aims is approached in a three-pronged fashion. We propose to use laser trapping technology to directly measure biomechanical and kinetic parameters of selectin bonds, use single cells on sparse substrates to understand the biomechanics of selectins in an in vitro flow chamber, and use intravital microscopy to study selectin biomechanics in the context of the living organism. We use molecular biology techniques to manipulate cDNA, cells, and mice to isolate each molecular mechanism. The insights gained from basic science-oriented studies are used to design liposome-based targeted drug delivery systems and ultrasound contrast microbubbles for delivery in the vascular system under shear flow.

STATUS OF RESEARCH AND PARTNERSHIP:

The partnership is in progress. We exchange reagents (antibodies, transfected cell lines) and technical expertise. The application of adhesion molecules for targeting purposes has been particularly successful. Therefore, Dr. Lindner was invited to become a full partner. We have organized the First Virginia Colloquium on the Biomechanics of Adhesion Molecules in 2000 and the second in 2001. Both were attended by approximately 50 scientists and graduate students. This year, Dr. Guilford will be organizing the Third Virginia Colloquium on the Biomechanics of Adhesion Molecules.

Recent publications:

1. Smith,M.L., Smith, M.J., Lawrence, M.B., Ley, K. (2002). Doubling plasma viscosity changes the rolling velocity of beads, but not of neutrophils. Circulation Research, in revision.
2. Lindner, J.R., Song, J., Christiansen, J., Klibanov, A.L., Xu, F., Ley, K. (2001). Ultrasound assessment of inflammation and renal tissue injury with microbubbles targeted to P-selectin. Circulation 104: 2107-2112.
3. Hafezi-Moghadam, A., Thomas, K.L., Prorock, A.J., Huo, Y., Ley, K. (2001) L-selectin shedding regulates leukocyte recruitment. J.Exp.Med. 193: 863-872
4. Park et al., April issue of Biophysical Journal.
5. Lindner, J.R., Song, J., Xu, F., Klibanov, A.L., Singbartl, K., Ley, K., Kaul, S. (2000). Noninvasive ultrasound imaging of inflammation using microbubbles targeted to activated leukocytes. Circulation, 102: 2745-2750.
6. Lindner, J.R., Dayton, P.A., Coggins, M.P., Ley, K., Song, J., Ferrara, K., Kaul, S. (2000). Noninvasive imaging of inflammation by ultrasound detection of phagocytosed microbubbles. Circulation 102: 531-538.
7. Lindner, J.R., Coggins, M.P., Kaul, S., Klibanov, A.L., Brandenburger, G.H., Ley, K. (2000). Microbubble persistence in the microcirculation during ischemia-reperfusion and inflammation: integrin- and complement-mediated adherence to activated leukocytes. Circulation 101: 668-675.

ISSUES:

Producing sufficient amounts of one of the molecules, PSGL-1, for in vitro studies continues to be a challenge. Recently, we have taken steps to obtain recombinant soluble PSGL-1.

PI: **LI, SHU-TUNG, PH.D.**
509 Commerce Street
Franklin Lakes, NJ 71417
T: (201) 405-1477
sli@collagenmatrix.com

PROJECT TITLE: Type I Collagen-Based Nerve Guide for PNS Regeneration

PARTNERS' NAMES AND AFFILIATIONS:
Frank Liuzzi, Ph.D., University of South Florida, Tampa, FL
Roger Madison, Ph.D., Duke University, Durham, NC
Julia Terzis, M.D., Ph.D., Eastern Virginia Medical School, Norfolk, VA

GRANTING NIH INSTITUTE/CENTER: National Institute of Child Health and Human Development (NICHD)

ABSTRACT:
This project is a collaboration of multidisciplinary fields of peripheral nerve repair and regeneration, involving Collagen Matrix, Inc., leading the BRP, specializing in extracellular matrix design and engineering; University of South Florida, specializing in neuroscience; Duke University, specializing in neuroscience, entubulation repair of peripheral nerve, clinical neurology and urology; and Eastern Virginia Medical School, specializing in neurosurgery.

The overall goal of this research partnership is to design, engineer and evaluate in vivo a type I collagen-based bioactive nerve guide for peripheral nerve regeneration applications.

The specific objectives of this project involve the isolation of the key design parameters and testing them in a rat sciatic nerve model. The final prototype, engineered from optimal design parameters, will be evaluated in two primate nerve models (median and cavernosal) as a potential entubulation repair method for clinical application.

In the first year we will isolate the design parameters and engineer the prototypes. In subsequent years, we will characterize the prototypes, test in vivo performance of the prototypes in a rat model, finalize the overall design, and finally, evaluate the final prototype in primate models. The overall program is designed for five years.

STATUS OF RESEARCH AND PARTNERSHIP:
Status of Research: This research summary covers work conducted from September of 2001 to February of 2002.
The specific aims 1 and 2 are designed to be performed in the first two years. They are: 1) design and engineer of resorbable, bioactive type I collagen-based nerve guide prototypes and 2) characterize the prototypes in vitro and in vivo.

The key design parameters include permeability of the tubular guide membrane, micro guiding channels within the tubular guide, inductive activity and adhesive activity of the tubular guide and the micro guiding channels. The progress of each of these design parameters is described below.

Permeability: We have engineered prototypes with various permeability properties. The permeability was controlled by the density of the membrane which was in turn controlled by the quantity of collagen fibers incorporated into the unit length of a nerve guide prototype of a given internal diameter and wall thickness. The method for analyzing the permeability has been developed. We used globular proteins of various molecular weights as probing molecules. We used the Bradford method of protein assay to determine the extent of probing molecules permeating through the nerve guide membrane. The probing molecules we chose include catalase (MW 250,000), bovine serum albumin (BSA) (MW 67,000), carbonic anhydrase (MW 29,000). The preliminary results showed that the prototype membrane can be controlled by varying the

density of the membranes and that a molecular weight cut off at the level of BSA can be achieved.

Micro-Guiding Channels: The method for engineering micro-guiding channels using filaments has been developed. The method for engineering micro-guiding channels using microtubes is under development.

Inductive Molecules: We proposed the use of two growth factors, basic fibroblast growth factor (bFGF) and insulin-like growth factor II (IGF-II), as a means to enhance the axonal growth. Both growth factors have been previously tested in animal models by other investigators and found to be effective in this application. Our approach has been to incorporate the growth factors into the walls of the device. Three technical hurdles must be overcome: the method of growth factor incorporation, activity of the growth factors in the composite state, and the effect of sterilization on the activity of the growth factors.

We first focused the research on the bFGF. We found that bFGF could be incorporated into the membrane structure using heparin as a binding mediator. That is, we first incorporated heparin into the collagen structure by a method we previously developed and then bind the bFGF to the collagen-heparin composite. We could bind 3 mg of bFGF for every gram of collagen-heparin composite. The activity of the bound bFGF was tested by culturing the baby hamster kidney cells (BHK-21, ATCC) in the presence of bFGF-incorporated membrane in 24 well culture plates, using collagen and collagen-heparin membrane alone as controls. Results of the cell culture studies showed that bFGF-incorporated collagen-heparin membrane enhanced the cell growth by a factor of 2 as compared to the controls.

We then tested the effect of γ-irradiation on the biological activity of the bound bFGF in the collagen-heparin composite. Gamma-irradiation at a dose of 25kGy (a dose used for sterilizing collagen products) has a substantial effect on the activity of bFGF. The activity reduced by about 35% of the controls using cell growth as a measure of biological activity. The remaining activity after gamma sterilization was more effective as compared to the matrix without the growth factor by about 50%. Since ?-irradiation is an effective and established method for the sterilization of collagen-based materials, we will test this bFGF-incorporated matrix as one of the potential materials in the rodent model. We will investigate other methods of sterilization to reduce the effect of sterilization on the growth factor.

bFGF-incorporated membrane matrices and controls have been sent to Dr. Frank Liuzzi at the University of South Florida for cell culture testing using PC-12 and Schwann cells as described in the proposal.

We will initiate research work of developing methods to incorporate IGF-II into the collagen-based matrix.

Adhesive Molecules: We proposed to use laminin as an adhesive molecule for axonal adhesion and growth. Laminin is known to be biologically active in this application. Since laminin has a heparin binding site, we believe that laminin will bind to heparin by described in the proposal. This work has just been initiated.

Conclusion of first seven months research work: The tools and resources are in place to conduct the proposed research work. We are making progress in the area we proposed for the first year and expect to accomplish the objectives set forth in the proposal.

Status of Partnership: Dr. Frank Liuzzi relocated from Eastern Virginia Medical School to University of South Florida. Therefore, the in vitro and in vivo rodent studies will be conducted at the USF facility. Dr. Julia Terzis at EVMS will be a consultant in the area of neurosurgery. Dr. Roger Madison at Duke University will be responsible for the primate studies. He is in the process of training primates in behavioral tests to measure functional recovery of the median nerve.

ISSUES:
None

PI: **LING, CLIFTON**
Deptartment of Medical Physics
Memorial Sloan Kettering Cancer Center
1275 York Avenue
New York, NY 10021
T: (212) 639-8301
lingc@mskcc.org

PROJECT TITLE: Multimodality biological imaging of cancer / tumor hypoxia

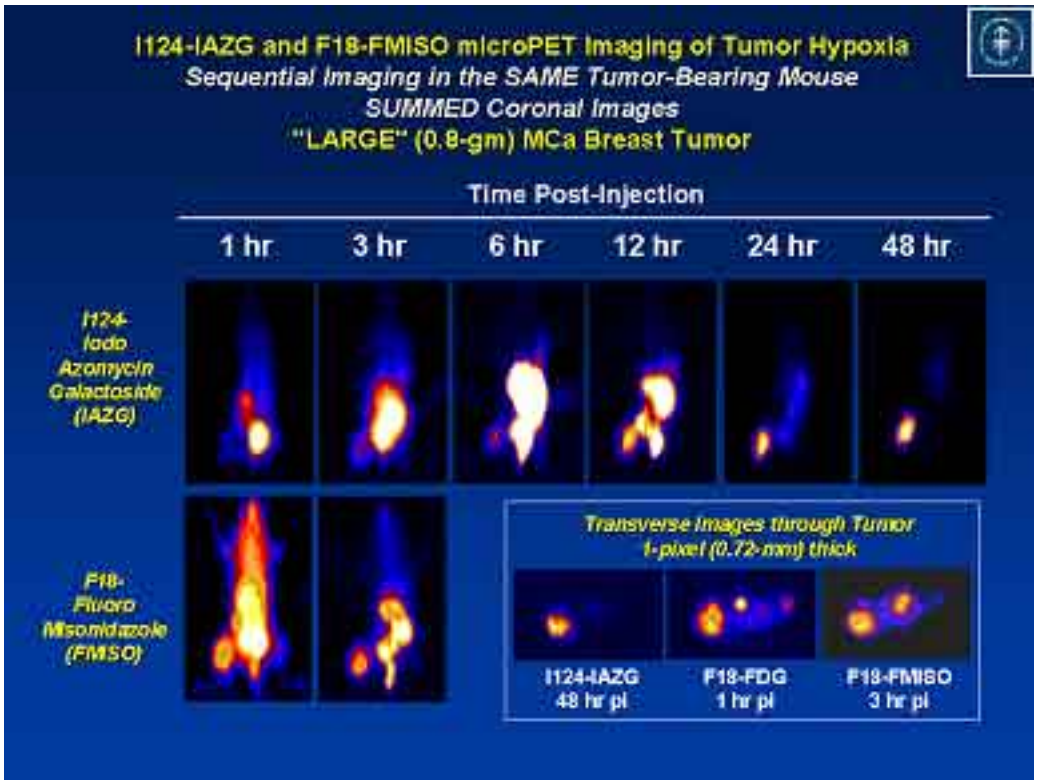
PARTNERS' NAMES AND AFFILIATIONS:
Ling, Koutcher, Humm, O'Donoghue

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

ABSTRACT:
Substantial progress has been made in 3 areas of the proposal, all of which have resulted in manuscripts in press.

(1) Measurement of pO2. Experiments were conducted using the OxyLite pO2 tissue oxygen tension sensor (Oxford Optronix, Oxford, England). The oxygen tension distribution was measured in two tumors; a spontaneous mouse fibrosarcoma, FSa-II, and a human squamous cell carcinoma xenograft, FaDu. The area where pO2 was equal to or lower than 2.5 mmHg was defined as the hypoxic lesion, and the hypoxic cell fraction (HCF) was taken as the fraction of these measurements in a tumor. The measured HCFs were compared with those determined by the paired survival assay for various sized tumors, and showed good agreement for tumors smaller than ~500 mm3. For tumors larger than ~500 mm3, the HCFs measured by this system were higher than those by the paired survival, indicating that the HCF measured by the oxygen probes included both hypoxic and necrotic areas in large tumors. We also evaluated the affect of continuous anesthesia (nembutal, or ketamine plus xylazine), on the measurements of pO2 and observed a consistent rise in the pO2 values during the first 20-30 min of measurements. Subsequently, the pO2 values became constant or continued to rise slowly.

(2) Imaging hypoxia by microPET. One of the principal objectives of the BRP proposal was the verification of non-invasive imaging surrogates of tissue pO2 and hypoxia. F18-FMISO studies have been evaluated as a PET marker of tumor hypoxia. IAZG is an alternative developed by our collaborator, Don Chapman, at the Fox Chase Cancer Center. I-124 is a novel long lived (4.2 day) positron-emitting isotope, which has been produced on the Memorial cyclotron and used to label IAZG. A representative set of microPET images from our animal imaging studies are presented in the figure below, comparing F18-FMISO (day 1) versus 124I-IAZG (day 2) in the same mouse. High uptake is observed in the large, but not the small MCa tumors. Tumor to background ratios of the tracer increase with time post injection. However, even as late as 6-8 hr, considerable F18-FMISO background activity clearly persisted in the liver and gut. Although, the tumor uptake of I124-IAZG was considerably slower than that of F18-FMISO, exhibiting poorer contrast than F18-MISO for times less than 6 hours, after 24 hrs and 48 hr pi, the liver and gut activity had decreased dramatically, and the tumor-to-background contrast for I124-IAZG was considerably higher than that for F18-FMISO. The results of this study demonstrate that superior hypoxia imaging becomes possible with longer-lived tracers such as I-124, on account of the ability to exploit the more rapid clearance from the normal non-hypoxic tissues.



(3) Imaging apoptosis by using iodinated-annexin V. Our goal in this investigation was to develop a method for iodinating annexin-V that would be suitable for the in vivo detection of apoptosis by SPECT and PET. Annexin-V was iodinated with 125I using two different techniques: by direct iodination with iodobeads resulting in the iodination of tyrosine residues, and via Bolton-Hunter reagent that binds to lysine. The active fraction of the labeled preparation was purified by affinity chromatography. We assessed thyroid accumulation of free iodide by comparing mice with blocked and unblocked thyroids We tested the ability of iodinated annexin-V to bind apoptotic cells in vitro using irradiated neuroblastoma cells, and in vivo using C3H mice subjected to total body-irradiation. When the iodobead preparation was injected into nude mice, activity rapidly accumulated in the thyroid within two hours, as observed by gamma camera imaging. By contrast, when annexin-V was labeled using the Bolton-Hunter protocol, there was no evidence of activity accumulating in the thyroid. The Bolton-Hunter labeled annexin-V bound to apoptotic cells in vitro. C3H mice were given 5 Gy whole-body irradiation. This treatment resulted in significant induction of apoptosis in the spleen, as measured by the TUNEL assay, and a four-fold increase in 125I activity in the spleens, relative to the controls. From this work, it can be concluded that direct iodination of annexin-V on tyrosine residues is a poor technique suffering from rapid de-iodination in vivo. With Bolton-Hunter chemistry one can produce a molecule that retains its label in vivo, and binds to apoptotic cells in vitro and in vivo.

STATUS OF RESEARCH AND PARTNERSHIP:

Our project was funded in October, 2001. The partnership has been working together in preliminary studies prior to that. The collaboration is now intensifying as the research project is being carried out.

ISSUES:

None

PI: **LIZZI, FREDERIC L., ENG.SC.D.**
Riverside Research Institute
156 William Street, 9th Floor
New York, NY 10038
T: (212) 502-1774
F: (212) 502-1729
lizzi@rrinyc.org

PROJECT TITLE: Integrated Ultrasonic Systems for Non-invasive Therapy

PARTNERS' NAMES AND AFFILIATIONS:
Weill Medical College of Cornell University, New York, NY
Columbia University College of Physicians and Surgeons, New York, NY
Spectrasonics Imaging, Inc., Wayne, PA

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI), National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
The ultimate objective of this 5-year Biomedical Research Partnership (BRP) is to develop a unified body of scientific knowledge and validated technology concepts that are needed to establish ultrasound as a practical non-invasive treatment modality and to inaugurate ultrasonic therapeutics as a new biomedical discipline. We will systematically elucidate the spectrum of ultrasonic therapeutic lesions that can modify various classes of diseased tissues, and we will develop integrated ultrasonic systems to position, induce, and monitor these lesions. We will focus on establishing a comprehensive basis for future treatments of cancer (primarily of the breast and prostate) and cardiac disease (primarily ventricular arrhythmia and myocardial insufficiency). These clinically significant diseases present challenging opportunities to test and refine our concepts, which have substantial implications for treating a broad array of problematic, life-threatening conditions.

This Biomedical Research Partnership involves biomedical engineering research at Riverside Research Institute; animal research studies at Weill Medical College of Cornell University (WMC) and Columbia University College of Physicians and Surgeons (CUCPS); and advanced systems development at Spectrasonics, Inc. Our multi-disciplinary research is designed to achieve a series of fundamental advances in the diverse areas involved in therapeutic ultrasound. We will employ extensive theoretical modelling to elucidate physical ultrasound-tissue interactions that can be used to produce therapeutic changes in diseased tissues. We will validate model results for thermal and mechanical effects in a series of animal experiments. Validated results will be used to design and implement advanced therapy systems incorporating ultrasonic arrays and real-time lesion monitoring. The systems will be tested and refined using animal experiments that investigate cancer and heart-disease therapy.

Our results will be incorporated in a systems model of ultrasonic therapy which will permit comprehensive treatment planning and design of future system features.

STATUS OF RESEARCH AND PARTNERSHIP:
The five-year program is now in its eighth month. All administrative arrangements among partners have been established, and a series of "kick-off" meetings have been conducted to set schedules and acquaint staff at each organization.

Studies are well underway, focused initially on producing thermal lesions in a controlled fashion to treat diseases by coagulating tissue. Riverside Research Institute (RRI) staff have conducted extensive computer simulations of the formation of thermal lesions and have designed candidate ultrasound beams that are optimized for specific types of treatment. They have also formulated a new means of monitoring induced therapy lesions by ultrasonically sensing motion induced by sub-threshold ultrasound exposures (via radiation force). This concept has been studied with comprehensive simulations of all relevant operations; initial experiments, utilizing a newly augmented laboratory system, have confirmed the feasibility of the technique.

Spectrasonics and RRI have specified a new system that integrates advanced therapy and imaging ultrasonic arrays for therapeutic aiming, exposure, and monitoring. This system is now being assembled and tested at Spectrasonics prior to its use in experiments at RRI.

Initial experiments for cancer therapy (Cornell U.) have focused on measuring tissue properties and verifying predictions of induced lesion size, shape and concomitant alterations in elastic properties. A cardiac group from Columbia U. visited RRI to perform initial experiments that documented the size and shape of non-invasively formed lesions in in-vitro myocardium specimens. Promising results led to the installation of an RRI laboratory system at Columbia for dedicated on-site cardiac studies.

ISSUES:

None.

PI: **LONG, RICHARD G.**
Department of Blind Rehabilitation
Western Michigan University
1903 West Michigan Avenue
Kalamazoo, Michigan 49008-5218
T: (616) 387-3451
Richard.Long@wmich.edu

PROJECT TITLE: Blind Pedestrians' Access to Complex Intersections

PARTNERS' NAMES AND AFFILIATIONS:
David Gutha, Paul Ponchilliaa, John Gesinkb,
Western Michigan University
a-Department of Blind Rehabilitation,
b-Department of Electrical and Computer Engineering

Duane Geruschat, Shirin Hassan,
The Maryland School for the Blind

Ron Hughes, David Harkey
The University of North Carolina's
Highway Safety Research Center

Dan Ashmeada, Rob Walla, Wes Granthama, Ken Framptonb
Vanderbilt University
a-Department of Hearing and Speech Science
b-Department of Mechanical Engineering

Randy Eastona, Billie Louise Bentzena, Janet Barlowb
Boston College
a-Department of Psychology
b-Accessible Design for the Blind

GRANTING NIH INSTITUTE/CENTER: National Eye Institute

ABSTRACT:
The pedestrian environment has become more challenging as transportation engineers have designed roadways to carry more traffic in less time. Wide arterial roads, actuated signalization, continuous flow designs such as slip lanes and roundabouts, and irregular intersection geometries are examples of intersection features that have made street crossings more challenging for pedestrians. This bioengineering research partnership of engineers, experimental psychologists, and rehabilitation professionals is working to improve access to complex intersections by pedestrians who are blind and visually impaired. The partners are engaged in research about basic perceptual and cognitive factors that affect orientation and mobility in complex pedestrian environments and the impact of various intersection design features on the safety and efficiency of pedestrians. These studies aid in understanding the nature of access problems that blind individuals experience. The team is also designing and evaluating new technologies for enhancing the orientation and mobility of persons who are blind as they negotiate complex intersections.

STATUS OF RESEARCH AND PARTNERSHIP:
The Western Michigan University and Vanderbilt University teams have documented problems in non-visual detection of vehicular gaps in traffic by pedestrians at roundabout intersections. The research

in this area, summarized at http://www.access-board.gov/news/roundabouts-bulletin.htm, has stimulated a great deal of discussion among traffic engineers and rehabilitation specialists since its publication in January, 2002. The amount and nature of the discussion highlights the need for further research concerning blind pedestrians' access to roundabouts, particularly the need for research that evaluates various approaches for enhancing access. The UNC team, supported by researchers at North Carolina State University, has recently begun a computer modeling/simulation project to aid in understanding how variables such as traffic volume and geometric characteristics (e.g., lane width and trajectory) affect distributions of gaps in vehicular traffic at roundabouts. The modeling effort will aid us in narrowing the options for intervention research. In future studies, automated pedestrian and vehicle detection technology will likely be explored in relation to pedestrian access at roundabout intersections.

The WMU team has completed engineering redesign of an anti-veering training device. This device provides feedback to blind users about their amount and direction of veer as they walk. The prototype will be available by the end of the project year for initial testing, and 15 units will be available by the end of 2002. The team also will begin data collection on a study of alignment to detectable tactile warning surfaces later this year.

The BRP teams at Vanderbilt University's Bill Wilkerson Speech and Hearing Center and Boston College are continuing their investigation of the relationship between the characteristics of accessible (audible) pedestrian signals (e.g., their location, intensity, sound characteristics) and their detectability/localizability. Like the roundabouts research described above, this research has stimulated discussion among engineers, blind individuals and researchers regarding ways to optimize pedestrian signalization. Studies have been conducted in both lab-based and outdoor settings. Installation of accessible pedestrian signals at intersections in four US cities will be accomplished early in Project Year 3. Data collection on various access issues before and after installation is planned for summer and fall, 2002. The results of this research will help guide the development of regulations concerning accessible pedestrian signalization that is currently underway.

The team at the Maryland School for the Blind is working collaboratively with researchers at the Wilmer Eye Institute of John Hopkins University. They are investigating the eye gaze and eye movement strategies of pedestrians with low vision as they negotiate complex intersections. Subject recruitment and data collection were the primary emphasis this year. Two experiments were completed. In the first experiment, 10 subjects with typical vision, 10 with macular degeneration, and 10 with glaucoma were required to cross at 2 complex and unfamiliar intersections while their eye gaze was measured. In the second experiment, 10 subjects with typical vision and 10 subjects with low vision were required to cross at 3 different intersections (simple plus, complex plus, and roundabout) while responding to a secondary task. The secondary task, a strategy used to evaluate the mental demand of a task, was the subjects' delay in responding to a randomly timed tone. Data analysis is currently underway for these eye gaze experiments.

The work of each of the teams is supported by a group of researchers at the University of North Carolina's Highway Safety Research Center. This team aids in developing collaborative relationships with traffic engineers throughout the US, and will assist in developing and disseminating results of the partnership's work to transportation engineers and policy makers. UNC is also working with NC State University on the application of simulation models to validate existing operational behaviors of visually impaired pedestrians at roundabouts and to develop alternative solutions for possible deployment in the field

ISSUES:
Evaluation, linkage between science and public policy development, linkages to researchers investigating other populations with similar concerns for access to the pedestrian environment.

PI: MAJUMDAR, SHARMILA, PH.D.
1 Irving Street, MRSC, AC 109
Department of Radiology, UCSF
San Francisco, CA 94143.
T: (415) 476-6830
Sharmila.Majumdar@radiology.ucsf.edu

PROJECT TITLE: Morphological and Functional Musculoskeletal Imaging

PARTNERS' NAMES AND AFFILIATIONS:

Dept. of Radiology, UCSF:
Sharmila Majumdar, Ph.D.
Thomas Lang,Ph.D.
David Newitt,Ph.D.
Katherine Andriole, M.D.,Ph.D.
Lynne Steinbach, M.D.
William Dillon, M.D.
Randall Hawkins, M.D.
Ronald Arenson, M.D.

Lawrence Berkeley National Laboratory:
Thomas Budinger M.D.Ph.D.
Ronald Huesman,Ph.D.
Henry van Broklin,Ph.D.

Dept. of Orthopedic Surgery, UCSF:
Jeffrey Lotz, Ph.D.
Michael Ries, M.D.
Serena Hu, M.D.
David S Bradford, M.D.
Edward Diao, M.D.
David Rempel, M.D.

Dept. of Medicine, UCSF:
Karen King, Ph.D.

Dept. of Neurosurgery, UCSF:
William Rosenberg, M.D.
Philip Weinstein, M.D.
Mitchel Berger, M.D.
Exponent Failure Analysis
Rajeev Kelkar, Ph.D.

GRANTING NIH INSTITUTE/CENTER: National Institute of Aging (NIA)

ABSTRACT:
In response to the announcement, PA number: PAS-00-006, participants from the University of California San Francisco (UCSF), Lawrence Berkeley National Laboratories (LBNL) and Industry (Focus Imaging, Exponent Failure Analysis, General Electric) propose to form a Bioengineering Research Partnership (BRP) focussed on the systematic study of the morphology and function of the musculoskeletal system in disease and health. In addition, resources from existing research relationships with General Electric Medical Systems, SUN computers and IBM will be combined and utilized to rapidly evaulate and disseminate the developments of the BRP. The aim of this consortium is to improve medical care through bioengineering developments, and to facilitate

close interactions between bioengineers, computer scientists, clinical investigators, basic scientists and corporate partners. This effort will expedite the development of clinically-relevant quantitative imaging tools and propel the technical advances from the laboratories into the operating rooms and clinics. We hypothesize that high resolution, fast magnetic resonance imaging techniques and positron emission tomography, combined with quantitative image analysis, processing and visualization, can provide new insights and clinically viable and relevant methods for objective evaluation of disorders of the musculo-skeletal system. The long-term objective of this partnership is to understand the link between morphology, function, biochemical changes and clinical symptoms in the musculo-skeletal system. An immediate objective is to develop, implement and optimize novel non-invasive imaging methods (magnetic resonance imaging: MRI and positron emission tomography: PET) that will allow us to depict the musculo-skeletal system, quantitate morphology, function, provide unique 3D visualization and graphical representations of function and morphology, as well as correlate these with biochemistry and c linical status. This research partnership is aimed at quantitating early degenerative changes in two clinical areas of emphasis: the knee and the spine. The first phase of the partnership will be technique development, followed by testing, and ultimately evaluation in case control studies in symptomatic patient populations. The specific goals are: (i) to develop quantitative morphological and functional markers for degenerative diseases of the spine, (ii) to develop quantitative morphological and functional markers for the degenerative changes in the knee and osteoarthritis.

STATUS OF RESEARCH AND PARTNERSHIP:

The research and partnership is progressing rapidly in some areas, and very slowly in others. While we have completed much of the requirements for year 1, in some specific aims, others are delinquent and have not even begun. Several manuscripts, research proposals will emerge from the successful arms of the partnership.

ISSUES:

The primary issue is maintaining and sustaining the communication between the partners.

PI: **MEANY, DAVID**
120 Hayden Hall
Department of Bioengineering
University of Pennsylvania
3320 Smith Walk
Philadelphia, PA 19104-6392
T: 215-573-3155
dmeaney@seas.upenn.edu

PROJECT TITLE: Molecular expression of force transmission in the central nervous system

PARTNERS' NAMES AND AFFILIATIONS:
Departments of Pharmacology, Neurosurgery, Genetics, and Bioengineering, University of Pennsylvania
Department of Neuropathology, University of Glasgow

GRANTING NIH INSTITUTE/CENTER: National Institute of Child Health and Human Develoment (NICHD)

ABSTRACT:
This bioengineering research partnership (BRP) brings together a broad team of bioengineers, neuroscientists, molecular biologists, bioinformaticists, and clinical scientists to examine the molecular substrates of traumatic brain injury (TBI). Long considered an untreatable disorder, recent scientific advances are now offering the potential to develop innovative strategies to treat and repair the traumatically injured brain. We now have, in hand, tools to replicate the mechanical forces experienced by the tissues during trauma, measure the resulting biochemical and genomic changes elicited by these forces, and we have a rapid means to evaluate therapeutics using in vivo animal models of central nervous system (CNS) injury. The focus of this BRP is to integrate these parallel advances across different fields to study the molecular expression of force transmission in the CNS. In this novel union of disciplines, this BRP can advance the rational evaluation and design of therapeutics to reduce the consequences of specific forms of brain injury. We target three exciting, strategic areas for fundamental advancement in this BRP:

Strategic area 1: Cell and tissue biomechanics in the CNS. The objectives in this area include (1) Developing quantitative estimates of tissue stress/strain generated in an in vivo model of traumatic brain injury, (2) mathematically modeling the stress transfer to cells within the CNS tissue during and applied macroscopic tissue deformation, and (3) formulating mechanical thresholds for neuronal necrosis and apoptosis using the neuropathological data obtained in Strategic Area 3.

Strategic area 2: Gene and protein expression profiling in mechanically injured CNS neurons: The objectives in this area include (1) merging robotics technology with the aRNA amplification technique to rapidly develop gene and protein expression profiles for three populations of neurons subjected to a defined mechanical stretch: those identified as (a) uninjured, (b) apoptotic, or (c) necrotic by immunohistochemistry, (2) refining and extending newly developed proteomics technology to scan simultaneously for proteins generated from mechanically injured neurons, comparing these proteins profiles to those in unstressed cells, and (3) using bioinformatics algorithms to develop a database that will correlate the transcriptional and translational changes with the applied neuronal cell stretch and with immunohistochemical data in treated and untreated animals generated in Strategic Area 3.

Strategic area 3: Evaluation of neuropathology and therapeutic strategies for traumatic brain injury: The objectives of this area include (1) defining the distribution and time-course of neuronal apoptosis and necrosis caused by mechanical stresses to neurons in the mouse cerebral cortex, used in Strategic Area 1 to determine injury thresholds, (2) selecting specific populations of neurons for expression profiling in Strategic Area 2, based on neuropathologic information and strain history, and (3) evaluating effect of death pathway-specific intervention strategies in vivo (AMPA receptor antagonists for apoptosis, NMDA receptor antagonists for necrosis) on the distribution of apoptotic and necrotic cells.

The central goal of this BRP is to identify the temporal, biochemical and genomic responses of neurons within the cortex exposed to well-defined mechanical conditions. This information will guide the development and evaluation of therapies that will augment the endogenous repair processes in the cortical neuron population. We reduce the complexity of studying the broad spectrum of traumatic brain injuries by focusing our efforts on a single common form of damage in brain injured patients - contusional damage to the cortex. Our long-term focus over the projected lifespan of this BRP is to apply the same infrastructure to design new injury-specific therapies for other important forms of brain injury, with the goal of reducing morbidity and mortality in head injured patients.

STATUS OF RESEARCH AND PARTNERSHIP:
The startup phase of the partnership is complete. Personnel have been hired to meet the needs of the partners. The scope has expanded to include in vitro techniques to mimic mechanical injury to the nervous system. These techniques will be used in addition to in vivo methods to assess the transcriptional and translational changes in neurons following mechanical injury.

ISSUES:
No significant issues have arisen during the startup phase of the partnership.

PI: **MITZNER, WAYNE**
Division of Physiology
Department of Environmental Health Sciences
The Johns Hopkins Bloomberg School of Public Health
615 N. Wolfe Street
Baltimore, MD 21205
T: (410) 614 5446
wmitzner@jhsph.edu

PROJECT TITLE: New Approach for the Treatment of Asthma

PARTNERS' NAMES AND AFFILIATIONS:
Broncus Technologies, Inc.

GRANTING INSTITUTE: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
This proposal seeks to further develop and evaluate an innovative and potential clinical treatment for asthma. Although there are a multitude of different possible triggers, an acute asthmatic attack is always characterized by contraction of the smooth muscle in the airway wall. Despite this common end point, most of the clinical asthma research and therapies in recent years have focused on understanding the immunologic factors that often lead to asthmatic attacks. In contrast, the present proposal describes research and development focusing on a treatment that will chronically impair the ability of smooth muscle to contract, and will thus be effective in treating asthma regardless of the initial trigger or intermediate pathways. It involves the design, construction, and application of a biomedical device that can limit the ability of the airway smooth muscle to narrow the airways. The work involves a close working partnership between the physiologic laboratories and expertise at the Johns Hopkins University and a biomedical engineering company, Broncus Technologies, that is providing the mechanical and bioengineering skills needed for product development.

As a means of treating asthma, Broncus Technologies, in collaboration with leading clinicians in the field of asthma, is developing a biomedical device system known as the AlairTM System (Pat. Pend.) The idea currently under development by Broncus Technologies involves delivering heat to the airway wall in a precise and controlled manner, such that the contractility of a significant fraction of the airway smooth muscle, in airways as small as 3 mm, is eliminated. The partnership established between Broncus Technologies and Johns Hopkins will facilitate the functional testing needed for this device development. In the long term, this partnership will help ensure the smooth translation of this new concept and device to the clinical arena.

STATUS OF RESEARCH AND PARTNERSHIP:
Since the initiation of funding in October of 2001, we have been setting up the logistics of experimental protocols to enable the treatment of experimental dogs and evaluation using computed tomographic imaging of the treated airways. The first goal is to more accurately confirm via CT the bronchoscopic preliminary data obtained by Broncus Technologies in treated airways. This will be done over the next 6- 12 months, and may involve modifications of the device to allow a broader setting of energy parameters. Once this validation work is completed, we will move to studying particle clearance from treated airways. In the process of impairing airway smooth muscle, the epithelium is transiently damaged, and one aim is to evaluate the dynamics of functional recovery of the ciliated epithelium.

ISSUES:
None at this time.

PI: OLSEN, DON B., D.V.M.
Director, Utah Artificial Heart Institute
803 North 300 West
Salt Lake City, UT 84103
T: (801) 323-1100
Don.B.Olsen@m.cc.utah.edu

PROJECT TITLE: Magnetically Suspended Rotor Blood Pump, 1 R01 HL64378-01

PARTNERS' NAMES AND AFFILIATIONS:
Utah Artificial Heart Institute
MedQuest Products, Inc.
University of Virginia

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

ABSTRACT:
The objective of this effort is to develop a novel ventricular assist device for patients suffering from congestive heart failure (CHF). The system will differ from current technology as follows:

1. A totally magnetically suspended impeller will be developed to eliminate contact bearings
2. A responsive physiologic controller will be developed to match system output to patient need without the use of sensors.

This system will provide the following benefits to CHF patients:

1. Significantly improved system reliability and durability relative to other devices in clinical use or in advanced development
2. Significantly improved patient quality of life by using the unique characteristics of the magnetic bearings to provide system output based on physiologic needs
3. Anticoagulation needs will be reduced, lowering the post-operative cost to the patient and reducing the likelihood of anticoagulation-related complications

The following specific objectives will be met:

1. A completely implantable continuous flow VAD configured for human use will be ready for transition to manufacturing
2. The system will include transcutaneous energy transfer (TET) system, batteries, and sealed implantable controller housing
3. One year in vitro reliability testing on complete pre-production systems will be completed
4. At least 6 complete system animal implants with a minimum of 60 day duration will be completed
5. The pre-clinical readiness Design History File and Design Review will be completed
6. A partnership with a commercial funding partner will be in place to prepare an Investigational Device Exemption (IDE) application, manufacture the system, and perform clinical evaluation

The continuous flow ventricular assist device (CFVAD) will be intended initially as a bridge to transplant, but the reliability characteristics will ultimately facilitate development of a longer term bridge to recovery and/or permanent device. By successfully completing this project, we can provide a superior treatment option at a lower cost for those who suffer from CHF. Developing novel means of addressing reliability and physiologic control issues will significantly improve the state of the art in circulatory devices, stimulating new development in acute circulatory support devices and in acute and chronic total artificial heart applications.

STATUS OF RESEARCH AND PARTNERSHIP:
Utah Artificial Heart Institute
There were 8 animal (calf) implants in year 02 of the contract. Three experiments were planned device fit trials in the design process of fitting the uptake cannula between the intraventricular cavity through the ventricular apex and the rotary pump chamber adjacent to the heart. The apex was opened with a coring device that leaves a hole through the apex of the left ventricular myocardium. The cannula was fitted with a sewing ring

to permit leak proof fixation between the titanium cannula after it was inserted into the ventricle and the ventricular myocardium. To avoid placing all of the experimental animals on a heart lung machine, the cannula placement and fixation was done on the beating heart. This procedure dictates several constraints. Two of the animals died of irreversible ventricular fibrillation while attempting/learning how to implant the pump. The cannula must be previously fixed to the pump chamber, which must be capped and primed with saline because the avoidance of any air in the system is crucial for the life of the animal.

Three animals were implanted with a working magnetically suspended impeller centrifugal pump. No measurable hemolysis was produced in these anticoagulated experiments. These calves lived for 13, 21 and 61 days. They recovered quickly from general anesthesia and the surgical procedure. They ate and drank water, gained weight and walked on the treadmill. The blood flow was very good in the longer surviving calf but the design and placement of the cannula resulted in limited blood flow in two of the calves. The only blood coagulation encountered in these experiments were found in the uptake cannula because of the design and material used.

University of Virginia
Computational Fluid Dynamics was applied to refine and to improve the design of the blood flow paths is the CF4 model of the HeartQuestä LVAD. The following tasks were completed: (1) Designed inlet elbow. (2) Designed outlet volute. (3) Optimized geometry of the hub and spindle. (4) Provided predictions of pump performance, moments and impeller torques in graphs and tables. (5) Performed a complete thermal analysis of the CF4. (6) Developed a transient simulation of the flow through the impeller, and predicted the transient forces on the impeller. (7) Developed a mathematical model of the human circulation system for testing physiologic control algorithms.

A significant problem that occurred in the pump was high axial forces due to fluid loads at flow rates in the 6 to 10 l/min range. The UVA research team designed a magnetic suspension system for the pump that had much higher axial load capacity to counter these high fluid forces.

With a plexiglass model of the CF4 provided by MedQuest to UVA, detailed measurements of the flow field within the CF4 centrifugal pump have been made with Particle Image Velocimetry (PIV) and Oil Streak Wall Shear measurements. The PIV technique has been used to make highly accurate measurements of the fluid velocity and turbulence characteristics in critical regions of the pump for both steady state and pulsed flow conditions, which model the physiological condition. An oil dot shear stress method was used to measure wall shear stresses in heart assist devices and applied to the same regions in the pump. This is a novel and relatively straightforward technique that will be used by many groups in the quantitative measurement of blood shear stress, which is critical in avoiding blood trauma. The measurements made with both these techniques have been used to understand the flow within the pump operating at design and off-design under steady flow and pulsed flow conditions and to provide a data set against which computational models may be validated and refined.

ISSUES:
MedQuest Products, Inc. has provided a pump to UVA and numerous pumps to Utah Artificial Heart Institute for animal testing. The partnership has made significant progress during this year and the partners are positioned to continue that progress.

PI: **PECKHAM, P. HUNTER, PH.D.**
Department of Biomedical Engineering
2500 MetroHealth Drive - Hamann 601
Case Western Reserve University
T: 216-778-3480
pxp2@po.cwru.edu

PROJECT TITLE: Development of Networked Implantable Neuroprostheses

PARTNERS' NAMES AND AFFILIATIONS:
Kevin L. Kilgore, Ph.D., Co-Principal Investigator
216-778-3801
klk4@po.cwru.edu

Biomec, Inc., Cleveland, Ohio
MicroStrain, Inc., Burlington, Vt.
Wilson-Greatbatch, Clarence, N.Y.

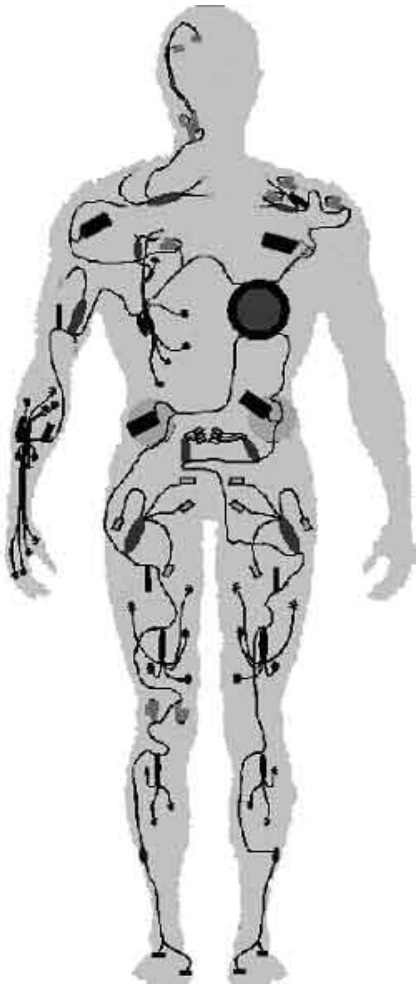
GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke (NINDS)

ABSTRACT:
Neuroprosthetic devices that electrically stimulate paralyzed muscles provide functional enhancements for individuals with spinal cord injury and stroke such as standing and stepping, reaching and grasping, and bladder and bowel function. Current implanted neuroprosthetic systems utilize considerable external powering and signal processing, and each system is tailored to the specific application for which it was intended. The need to design a customized implant system for each application severely limits progress in the field and delays introduction of new technology to the end user. Therefore, we propose to design, fabricate and evaluate an implanted neuroprosthesis with an open architecture that can be easily configured for current and anticipated neuroprosthetic applications, allows accommodation of new innovations by various participants in the field, minimizes external components, and can be clinically implemented.

The implant design we propose is based on a network of small implanted modules, distributed throughout the body. Each module will contain local processing capabilities in order to minimize the communication rate between modules, and will be programmable through a transcutaneous link. The initial configuration of the network will use leads to connect each module, but the network design and communication protocol will be developed in anticipation that each module may, in the future, communicate with other modules through a wireless "intra-body" network. Primary powering will be provided through a single transcutaneous RF link, which is connected to the distributed modules through single low-resistance multi-conductor leads, and each module will contain a secondary internal power supply. A variety of modules will be developed, each with a specific function including: muscle-based stimulation, nerve cuff stimulation, biopotential (electromyogram, electro-oculogram, electro-encephalogram, electroneurogram) signal recording, body segment orientation measurement and acceleration measurement. Other potential modules that could be incorporated into this system include mechanical actuators, joint angle transduction, and strain gage based sensors. In order to develop a system that is both clinically relevant and commercially viable, we have assembled a partnership between representatives from industry, academia, health care and consumers.

STATUS OF RESEARCH AND PARTNERSHIP:

Our partnership was established in October, 2001. We have evaluated various configurations of our networked structure, and have selected our primary design configuration. This configuration, shown in the figure, utilizes standard network and communication protocols to the maximum extent possible. This approach allows us to benefit from advances in technology that will transpire over the lifetime of our technology. This configuration will enable multiple modules (described above) to be added to the network in a flexible manner to meet a wide variety of clinical needs.



From a management perspective, we have established an independent "tiger team" in our laboratory, which is focused exclusively on the conduct of this project. We have developed the contractual relationships with our partners, and are currently refining their work scopes, priorities, and deliverables. We have also established a bio-power team in conjunction with the Electrochemistry Center on our campus. The bio-power team is evaluating powering options for the system, both near term and long term, and has retained the participation of a distinguished retired industrial consultant to facilitate discoveries and provide entrée to new partners.

ISSUES:

Our primary issue is managerial rather than technical. We feel that we have made good progress in the research. However, our university is rather less sophisticated in enabling flexible relationships with subcontractors that would enable us to add and terminate relationships based upon performance and new discoveries. Flexibility apparently would require a variance in the NIH regulations.

PI: PELI, ELI, MSEE, OD
Schepens Eye Research Institute
Harvard Medical School
Schepens Eye Research Institute
20 Staniford Street
Boston MA 02114-25081
T: (617) 912 2597
eli@vision.eri.harvard.edu

PROJECT TITLE: Engineering Approaches to Low Vision Rehabilitation

PARTNERS' NAMES AND AFFILIATIONS:
Bitflow Inc., Woburn, MA
Massachusetts Eye & Ear Infirmary, Boston, MA
Boston College, Chestnut Hill, MA
DigiVision Inc. San Diego, CA
The Lighthouse Inc., New York, NY
Chadwick Optical, VT
University of Cambridge, England
MicroOptical Corp., Westwood, MA
National Advanced Driving Simulator, Iowa City, IO

GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

ABSTRACT:
This project applies novel engineering approaches to the problems of low vision rehabilitation. We are building prototype devices based on solid theoretical foundations that, eventually, will become marketable rehabilitation products. The devices, designed and built with the help of our engineering partners, will be tested critically using diverse patient populations, with the help of the clinical partners to determine the effects on function and on the quality of life.
We shall develop and test both optical and electronic devices that implement three specific engineering approaches aimed at restoring (at least in part) the important interplay of central (high-resolution) and peripheral (wide-field) vision. The three engineering approaches that we will explore are multiplexing, dynamic control of display, and image enhancement. Also, we will show that various combinations of these approaches are possible and likely to be beneficial. In our assessment and testing we emphasize two approaches: a virtual environment for controlled and quantitative testing in the laboratory, and on-the-street evaluation for real-life determination of the effect and usefulness of the devices and techniques.

STATUS OF RESEARCH AND PARTNERSHIP:
The project has two major components: device development and device evaluation. Both components have been progressing well. Work has moved forward on the three mobility aids: a paper describing the principle of visual multiplexing has been published; progress on the development of the two optical devices is significant; multiple prototypes of the electronic device, augmented-vision for monocular restricted peripheral visual field, have been implemented and tested, and a paper submitted. A study of eye movements of patients with tunnel vision was completed, providing useful information on the required field of view of the HMD (augmented-vision system); the design of the second generation of the HMD was specified and system is under development; the portable edge detection and stationary image enhancement devices have been delivered in prototypes. Another novel optical device using multiplexing was invented and a patent

application submitted. The systems for the implementation of the dynamic control of displays, that will be used to enhance television viewing by people with central visual field loss, were delivered and are being assembled for initial testing. The virtual Mall is in place and has basic functionality. Further developments of that system are progressing well. The real walking study is ready and waits better weather in Boston. Recruitment of subjects is in progress.

We have published in the last year 5 journal papers, 4 conference proceedings and a book related to the project. We have presented numerous conference papers including invited and award presentations on the subject. An active web site serve both for internal project communications and dissemination of information.

ISSUES:
The National Advanced Driving Simulator (NADS) development is about 2 years behind schedule in Iowa. While the facility is almost complete now, it appears that it may take a while longer before studies with patients on a regular basis can be conducted. We have been exploring a number of alternative options. We have filed a Technology Enhancement Grant application to obtain a smaller, simpler driving simulator for our lab. We conducted a pilot testing with three patients on such a simulator. If this is awarded we will be able to conduct the study at our location. We continue to work with the NADS team in case progress there is faster than we currently anticipate.
The local Department of Motor Vehicles rejected the hemianopia driving study. At the same time a similar study without the treatment component of our study was concluded in Holland. We formed a new partnership with the group from Groningen, Holland and made arrangements to switch the study site there. All protocol and IRB issues are resolved. We await State Department approval for an overseas study.

One of the partners, BitFlow, was unable to provide the manpower needed for the development of one of the components. We were lucky that another partner, DigiVision, was able to step in and provide an alternative development which was delivered.

We have a formed a new partnership with Chadwick Optical from Vermont (in place of the Swedish partner, Multilens), that proved very effective and aggressive in delivering a number of improvements in two optical systems.

PI: RABBIT, RICHARD D., PH.D.
2480 MEB, Bioengineering
50 South Central Campus Drive
Salt Lake City, UT 84112
T: (801) 581-6968
r.rabbit@utah.edu
http://www.bioen.utah.edu/faculty/RDR

PROJECT TITLE: Micro-electric impedance spectroscopy (μEIS) of hair cells

PARTNERS' NAMES AND AFFILIATIONS:
Baylor College of Medicine (W.E. Brownell)
Georgia Institute of Technology (A.B. Frazier)
NASA Ames (R. Boyle)
University of Utah (R.D. Rabbitt)
Washington University (S.M. Highstein)

GRANTING NIH INSTITUTE/CENTER: National Institute of Deafness and Communication Disorders (NIDCD)

ABSTRACT:
This project is aimed at the development and testing of micro-electric impedance spectroscopy (μEIS) and tomography (μEIT) hardware and reconstruction software to record and image the spatio-temporal distribution of electrical properties within the cytoplasm, organelles and membranes of vestibular and auditory sensory hair cells. A combination of flex-circuit technology and standard lithographic microfabrication techniques are used to construct micro-recording chambers instrumented with arrays of metal electrodes at subcellular dimensions. Isolated cells are positioned within the instrumented recording zone under mircroscopic observation and interrogated using radio frequency electrical signals. Voltage and current are measured around the outside surface of the cell and used to reconstruct three-dimensional maps or images of the conductivity and permittivity throughout the cell. μEIT systems are being used to interrogate electrical properties of cochlear outer hair cells and type II vestibular hair cells in response to micromechancical stereocilia displacements, electrical stimuli, and acetylcholine efferent neurotransmitter stimulation. Results are contributing to our fundamental understanding of the spatial distribution and temporal response of electrical properties in these important sensory neurons. Perhaps more importantly, μEIT devices developed as part of the research, are providing an entirely new window through which to view the living machinery of a wide variety of normal and pathological cells. The project integrates bioelectricity, imaging, bioinstrumentation, micro/nano-bioesensors, physiological modeling/computation, biomechanics and microfluidics. Devices involve on-chip transport of solutions/pharmaceutics and living cells.

STATUS OF RESEARCH AND PARTNERSHIP:
The project is currently in the 7th month of the first year of funding (1 RO1 DC04928-01, start date: August 2001). All subcontracts were established within the first month of the grant. The project is proceeding as outlined in the proposal. Experiments using simple two-electrode μEI devices with saline solutions and cells have provided additional data and have contributed to incorporation of an on-board RF computer-controlled bridge circuit in the 2nd generation devices. We have completed masks for two different 2nd generation μEI platforms and are currently in the fabrication stage. Due to the small scale of the devices and high interrogation frequencies employed, considerable attention has been devoted to the development of reliable, user friendly, microfluid and electrical

interconnects. Over the next few months we will interface these new μEI devices to a bank of computer controlled arbitrary waveform generators and digital scopes for testing (hardware and software). Animal protocols are in place and we will begin μEI testing of cochlear outer hair cells and vestibular hair cells using the 2nd generation devices this spring. Initial experiments will measure the time course of the prestin-associated charge movement in the lateral wall of the cochlear outer hair cells at temporal resolution far exceeding that possible with pipette-based recording technologies. These data are expected to play an important role in the identification of ions and/or charged domains of prestin associated with nonlinear capacitance modulation in outer hair cells - biophysical events fundamental to outer hair cell somatic electromotility and the exquisite selectivity and sensitivity of the mammalian cochlea.

ISSUES:

We have not experienced any serious administrative or technical issues. BRPs, like all projects involving numerous institutions, are bound to experience administrative delays in establishing subcontracts and generating reports - but we have been able to deal with these challenges through advanced planning and a little flexibility in project timing. I do have a concern regarding technology transfer. Our research is driven by specific set of scientific questions - questions which require the development of new technology to answer. The PI is concerned that efforts by the team members in the area of technology transfer may dilute the time available to devote to the key scientific questions at hand. Technology transfer is typically a very poor use of the expertise of senior scientists. Would it be wise to support technology transfer efforts, where appropriate, under completely separate grants?

PI: **RATNER, BUDDY D.**
University of Washington
Box 351720
Seattle WA 98195-1720
T: (206) 616-9718
ratner@uweb.engr.washington.edu

PROJECT TITLE: Engineered Cardiac Morphogenesis: Stem Cells and Scaffolds

PARTNERS' NAMES AND AFFILIATIONS:
Advanced Tissue Sciences, Inc., La Jolla CA
Prof. Kim Woodhouse, Univ. of Toronto, Toronto Ontario CANADA
Drs. Robert Vernon and Margaret Allen, Hope Heart Institute, Seattle WA
Dr. Jorge Heller, Applied Pharma, Redwood City, CA

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
The long term aims of this project are to produce tissue engineered ventricular wall patches for myocardial repair, ventricular assist devices, and eventually replacement ventricles. Our team from academia and industry has expertise in biomaterials, bioreactors, tissue biomechanics, embryonic and somatic stem cells, muscle development, vasculogenesis, extracellular matrix, cardiac injury and regeneration, animal and human heart transplantation. This team will collaborate across three research foci: **1) "Instructive" tissue scaffolds.** Advanced biomaterial fabrication will be used to engineer biodegradable matrices and meshes with controlled pore dimensions, modified with receptor specific molecules. Matrices will be optimized to instruct cell attachment, orientation, migration, proliferation, differentiation, and overall tissue organization. **2) Cell and developmental biology.** Primary and stem cell-derived muscle and vascular cells will be studied on modified scaffolds to determine the optimal conditions for producing functional muscle tissue and vascular networks. Engineered tissues will be subjected to mechanical stresses to direct maturation toward in vivo phenotypes. Bioreactors will be developed to implement these requirements on a useful scale. **3) Clinical science and animal models.** Contractile ventricular patches will be tested in an injured heart model. Integration with host tissue and restoration of contractile function will be evaluated. A tubular cardiac assist organ comprised of vascularized myocardium and endocardium will also be developed. The "tube hearts" will be conditioned in pulsatile flow circuits, assessed for mechanical performance in vitro, and eventually grafted into aortas of syngeneic rats for in vivo evaluation. Progress toward these goals should establish design principles necessary for constructing more complex ventricular devices

STATUS OF RESEARCH AND PARTNERSHIP:
This BRP comprises six major laboratories at the University of Washington, two at the Hope Heart Institute, one at the Univ of Toronto, and laboratories at Advanced Tissue Sciences as well as collaborations with Advanced Polymer Systems. The program is currently in its second year of funding.
Status of the Partnership: The partnership is active and robust. The UW laboratories are highly interdisciplinary and include units from both the College of Engineering and the School of Medicine. The participation of the Hope Heart Institute in this program has been enthusiastic. The partners from Toronto, San Diego, and San Jose continue to willingly expend the time, effort and money to travel to Seattle for strategic planning meetings and research.
Status of the Research: The project has three broad concerns which support an overall goal of achieving a tissue engineered ventricular patch: 1)advanced tissue engineering scaffolds, 2) basic research in cell and developmental biology, and 3) animal/clinical models. Within these three area are ten specific aims. For the most part, work in this second year has concentrated on specific aims dealing with materials, cell biology and engineered construct and bioreactor prototypes Some animal work has been contributed,

however the bulk of the animal studies are just beginning due to the relocation of an investigative team to a new institution.

Previously we reported on a system for controlling proliferation and differentiation by controlling the induction of FGFR1 via an exogenous ligand in a skeletal myoblast system. This work has progressed: activation of the MAP kinase pathway was demonstrated, the effects were proven to be reversible, and the experiments were extended to an in vivo environment. This work was recently published in J. Biol. Chem. We now are moving to apply this system in ES cells. Cell selection from mouse ES cell embryoid bodies: This work continues as the cell type specific promoters brachyury (mesoderm) and cardiac alpha-actinin (cardiomyocytes) are being cloned with fluorescent protein markers for use in cell sorting. Scaffold Testing and Seeding: We have acquired a rotational bioreactor (HARV) and begun several scaffold seeding experiments with novel scaffold materials. Additionally mouse implantation experiments are being used to screen materials for toxicity to the myocardium.

Previously we reported on the fabrication of three-dimensional scaffolds with defined pore size based on poly (HEMA). These materials have been used now for in vivo implant studies, cell seeding studies, and in vitro studies with endothelial cells. In general, cell attachment and survival on these materials has been poor. Thus we are pursuing strategies to include ECM proteins with the scaffolds to improve cell attachment. Work is also progressing on the synthesis of a biodegradeable cross linker for this system. Our work with degradeable polyurethanes has progressed as we now fabricate porous foams from this material. These scaffolds are now in cell seeding studies. We are also beginning experiments with a new decelluarized matrix based scaffold system.

To better understand the developmental processes that lead to cardiac morphogenesis, we are employing RT-PCR to study growth and transcription factors in pluripotent P19 embryonal carcinoma cells. In addition, our studies of the conversion of E3 chick cardiomyocytes to Purkinjie fiber cells is continuing. We are also studying the transcription factors associated with the TREX control element involved in cardiac gene regulation, with an aim to potentially improve long term cell function via gene therapy.
Previously we have reported on the manufacture of spun (drawn) and electrosprayed microfibers for use as scaffolds. Presently work has focused on drawn fibers of polypropylene arranged in parallel arrays. C2C12 myoblasts have been cultured on these arrays and, with a fiber spacing of 20-55 microns, form aligned muscle cell sheets that, when differentiated, form myotubes and contract in culture. Experiments to stack these arrays in the third dimension are planned.

Addressing angiogenesis and vasculogenesis in engineered tissues continues to be a concern and research focus. Also, our previous work on two-dimensional patterning of cells has largely given way to novel approaches for the entrapment of cells in three-dimensional hydrogels - work which is still in its early stages.

Work done by our corporate partner Advanced Tissue Sciences, includes further studies of ES cells on commercial scaffolds (Biobrane, TransCyte) and characterization by gene array analysis. Several bioreactor designs are progressing through the prototype stage.

ISSUES:
Most of the challenges of the program have involved the normal issues associated with starting and administering a large inter-institutional research program. Staging of the research (i.e. progress in one area being dependent upon completion of advances from another area) has proven to be a bit of a concern. Mechanisms for dealing with intellectual property exist, but are cumbersome and drawn out. The travel cost to ensure a healthy and participatory interaction between the partners has been significant, and these costs have largely been borne from outside the NIH funding. Finally, although we propose no human species work at this time, we forsee the recent decisions on federal support of stem cell research having a significant impact on the work of our field in general.

PI: **RENSHAW, PERRY F.**
McLean Brain Imaging Center
115 Mill Street
Belmont, MA 02478
T: (617) 855-3750
perry@mclean.harvard.edu

PROJECT TITLE: High Field MR Research in Drug Abuse: A Bioengineering Research Partnership

PARTNERS' NAMES AND AFFILIATIONS:
Brain Imaging Center, Behavioral Psychopharmacology Research Laboratory, Developmental Biopsychiatry Research Program, McLean Hospital, Belmont, MA
Bioengineering Center, Department of Electrical Engineering and Computer Science, Tufts University, Medford, MA
Department of Psychiatry, Boston University School of Medicine, Boston, MA
Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine

GRANTING NIH INSTITUTE/CENTER: National Institute on Drug Abuse (NIDA)

ABSTRACT:
Magnetic resonance spectroscopy (MRS) and functional magnetic resonance imaging (fMRI) are extraordinarily promising new imaging modalities that are increasing our understanding of the nature of drug abuse and addiction. In March, 1999, the Office of National Drug Control Policy (ONDCP) and McLean Hospital agreed to jointly fund a Varian NMR Systems 4.0 T MR scanner which will be dedicated to substance abuse research at the McLean Brain Imaging Center.

The present BRP application describes a series of ten engineering projects which will enhance the capabilities of this unique magnetic resonance research center to conduct studies of individuals with substance abuse disorders. This research program will involve bioengineering and clinical investigators at McLean Hospital, the Beth Israel Hospital, Tufts University, Boston University, the University of Washington, the University of Oxford, the University of California, San Francisco, and Wayne State University.

Specific projects are summarized below:

1. Objective motion detection and correction in time series fMRI experiments.
2. Optimized phased array coil design.
3. FMRI image registration and signal dropout reduction in brain regions with high susceptibility effects.
4. Functional T2 relaxometry of brainstem and midbrain monoaminergic nuclei.
5. Estimation of cerebral blood flow and volume using dynamic susceptibility contrast MRI.
6. Proton echo-planar spectroscopic imaging at 4 T.
7. Two-dimensional, proton magnetic resonance spectroscopy of amino acid neurotransmitters.
8. Statistical methods for assessing drug effects and confounds in MRS and fMRI studies.
9. Concurrent, high resolution optical imaging and fMRI.
10. Concurrent EEG and fMRI assessment of drug-induced alpha wave activity.

All of the projects listed above have been have been designed to address technical limitations

encountered in the course of conducting NIDA-funded clinical imaging studies at 1.5 T field strength. Importantly, funds requested for this BRP will be used exclusively to support the engineering aspects of the research projects.

STATUS OF RESEARCH AND PARTNERSHIP:

1. The Varian Unity/Inova 4 T Scanner was installed at McLean Hospital in May, 2001.
2. The scanner quenched on two separate occasions in July and in August; this was ultimately attributed to wiring problems within the scanner.
3. Our BRP grant was funded by NIDA, with a substantial budget cut, effective 1 September 2001. Of the ten projects listed above, one (#8) was eliminated and two (#2 and #3) were combined. The remaining 8 projects all had significant budget cuts, based primarily on the review of the grant proposal.
4. Additional funding to support hardware purchases was obtained from the Counterdrug Technology Assessment Center (CTAC) of the Office of National Drug Control Policy (ONDCP) to expand the scope of the work that we could do within the BRP. Equipment has been ordered and some has been delivered. Work on projects #9 and #10 has been delayed due to long lead times for equipment delivery.
5. Staff for two projects (#5 and #6) had to be recruited and hired. This has now been accomplished.
6. A research agreement was established with OPTAx, Burlington, MA to supply two near infrared, high resolution cameras for project (#1).
7. Methods for fully implementing 2D MRS on the 4 T scanner have been reduced to practice (project 7).
8. We continue to work with Varian to improve the performance of the scanner for human clinical studies. Unresolved issues include: comfort and performance of the patient table; software and filters for decoupling; EPI stability and ghosting; user interface; and sound dampening.
9. Two new areas of research: carbon-13 MRS studies of cerebral metabolism (in collaboration with investigators at Yale University) and biological effects of magnetic stimulation have been identified.

ISSUES:

1. Mechanisms for expanding the scope of the research to involve new Partners are not entirely clear to us.
2. We have a strong preference for this BRP remaining with the original funding agency (NIDA) rather than being transferred to NIBIB.

PI: **RYLANDER, H. GRADY RYLANDER**
The University of Texas at Austin
Eng Sci Bldg R617C
Austin, Texas, 78712
T: (512) 471-1995
F: (512) 471-0616
rylander@mail.utexas.edu

PROJECT: TITLE: Polarization Sensitive Optical Coherence Tomography (PSOCT) for Glaucoma

PARTNERS' NAMES AND AFFILIATIONS:
Johannes de Boer, Barry Cense, Teresa Chen, Hyle Park
Harvard Medical School
Wellman Laboratories of Photomedicine, Massachusetts General Hospital

GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

ABSTRACT:
The goal of our project is to measure depth resolved birefringence of the retinal nerve fiber layer (RNFL) and study how the birefringence changes with glaucoma. We have constructed two PSOCT systems: an open air PSOCT system at UT Austin to evaluate cynomologous monkeys and a fiber-based PSOCT system at Massachusetts General Hospital to evaluate human subjects. A typical measurement of phase retardation/unit depth (PR/UD) in the retina between the fovea and optic nervehead of a human volunteer at Massachusetts General Hospital is shown in figure 1. A typical measurement of PR/UD in the superior arcuate bundle of a cynomologous monkey is shown in figure 2. The initial slope of these graphs indicates phase retardation within the RNFL at one retinal location. These are the first in vivo depth resolved measurements of monkey and human RNFL birefringence. Both measurements yield an average PR/UD of 0.3 degrees/micron which compares well with the PR/UD = 0.2 degrees/micron reported by Knighton in the rat and 0.1 degrees/micron reported by Weinreb in cynomologous monkeys. The PR/UD is not constant across the cynomologous retina but varies between 0.1 and 0.3 degrees/micron depending on where the measurement is taken. This observation implies that birefringence depends on RNFL morphology. RNFL thickness cannot be determined from a measured phase retardation without knowing the PR/UD at that specific location.

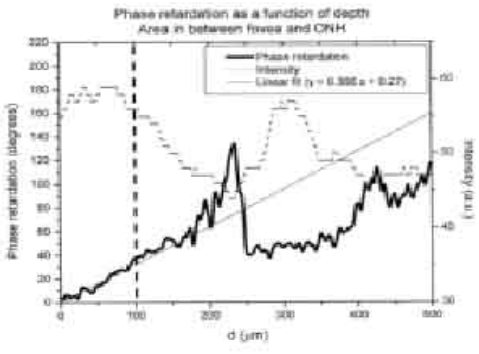


Figure 1: PR/UD for human.

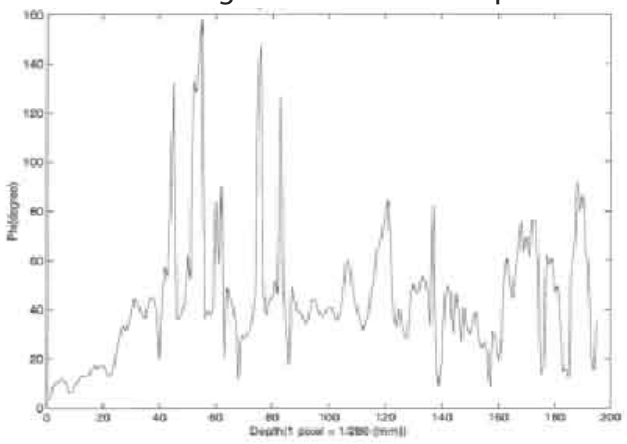


Figure 2: PR/UD for Cynomologous Monkey.

To measure the functionality of the RNFL in vivo, a differential phase optical low coherence reflectometer (OLCR) has been built that can resolve one milliradian or an optical pathlength difference of one angstrom. This resolution is theoretically sufficient to image the birefrinbence change produced by an action potential on a single neuron with signal averaging.

Status of Research and Partnership: The partners have developed complimentary solutions to measure depth resolved phase retardation in the peripapillary RNFL. The UT Austin group is implementing a rapid scanning optical delay line (RSODL) so that complete 3D maps of PR/UD can be constructed over a 6mm X 6mm area of the peripapillary retina in 10 seconds. We plan to measure these PR/UD maps in a group of monkeys with monocular glaucoma to determine the registration robustness in sequential images over time and the changes in the PR/UD maps as a result of glaucoma progression. Our working hypothesis is that a decrease in PR/UD will be an early metric for loss of retinal ganglion cells. Retinal histology will be compared with the birefringence maps to associate axonal loss with a change in PR/UD. The Massachusetts General Hospital group is using a slit lamp interface to their PSOCT instrument to determine a normative human database for the PR/UD maps and then study glaucoma patients. Comparison between normal and glaucoma subjects will be used to develop algorithms to discriminate glaucoma and normal eyes and assess PSOCT measures of glaucoma progression in humans. The OLCR will be tested on the RNFL to determine if there is amplification of the birefringence signal due to simultaneous sampling of many active neurons.

ISSUES:

1. Change in research focus as the project evolves
2. Delays caused by technical problems
3. Communication between partners
4. Technology transfer to industrial partners
5. Guidance for clinical trials
6. Transfer of the project from NEI to the Imaging Institute

PI: **SACKELLARES, J. CHRIS, M.D.**
University of Florida
PO Box 100244
Gainesville, FL 32610-0244
sackellares@epilepsy.health.ufl.edu

PROJECT TITLE: Bioengineering Research Partnership for Brain Dynamics

PARTNERS' NAMES AND AFFILIATIONS:
University of Florida: J. Chris Sackellares, M.D.; P. Carney, M.D.; J. Principe, Ph.D.; P. Pardalos, Ph.D; S. Roper, M.D.; M.Yang, Ph.D.; J. Harris, Ph.D; R. Melker, M.D., Ph.D.; H. van Oostrom, Ph.D.
Arizona State University: L. Iasemidis, Ph.D.

GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke (NINDS)

ABSTRACT:
Epilepsy is a common neurological disorder that causes spontaneous recurrent seizures. In spite of major advances in pharmacology, neuroimaging, clinical neurophysiology, and neurosurgery, many patients remain disabled due to uncontrolled seizures. We propose to develop novel diagnostic and therapeutic tools, based on recent discoveries regarding dynamical mechanisms initiating epileptic seizures. We have found characteristic preictal dynamical changes, detectable in the electroencephalogram (EEG), preceding seizures by over 30 minutes (preictal transition, PT). More recently, other investigators have confirmed the presence of PDT. Our research indicates that the PT is demonstrable in the EEG in approximately 90% of seizures and that automated paradigms can be used to predict seizures. The potential to predict seizures in advance provides an opportunity to develop innovative diagnostic and therapeutic approaches. Our specific aims are: *(1) Specific Aim 1. To continue the development of dynamic measures for the quantification of the spatiotemporal properties of the epileptic transition (years 1-3); (2) To develop specific pattern recognition algorithms for a seizure warning system (SWS) based upon the on-line features of the dynamical properties of brain electrical activity (years 1-4); To implement the dynamic features and pattern recognition algorithms in a SWS for on-line, real-time detection of the preictal dynamical transition (years 2-4); and (4) To evaluate the effects of therapeutic interventions during the preictal transition (years 1-5).*

The specific spatiotemporal patterns of the PT vary from seizure to seizure and patient to patient. Thus, sensitive and reliable SWS will require sophisticated signal processing techniques. Dynamical measures will be augmented by other powerful analytic approaches, including multivariate time-series analysis, pattern recognition algorithms, and optimization techniques. To this end, we have gathered experts in signal processing, optimization, V.L.S.I., neurophysiology, neuroanatomy, epilepsy, and neurosurgery. The work will involve the coordination of several research sites throughout the University of Florida Campus including the Brain Dynamics Laboratory (Malcolm Randall V.A. Medical Center), Computer NeuroEngineering Laboratory College of Engineering), Center for Applied Optimization (College of Engineering), an In vitro Neurophysiology Research Laboratory (University of Florida Brain Institute), an In Vivo Neurophysiology Laboratory (Department of Pediatrics) and the Epilepsy Monitoring Laboratory (Shands Hospital). We anticipate that the proposed efforts will result in prototype diagnostic software and devices by the end of year 5. We also will obtain preliminary data that will be used for the design and testing of implantable devices that will activate pulsed therapeutic interventions during the preictal transition.

STATUS OF RESEARCH AND PARTNERSHIP:
The BRP grant award in July 2001 enabled us to establish an administrative and laboratory core, thus enhancing productivity of an established research team. Research is conducted in each of 5 laboratories. Work groups meet on a daily basis. Weekly technical meetings, involving the entire partnership, provide a forum for each work group to present progress to others and obtain feedback. Weekly research seminars provide a forum for academic multidisciplinary presentations on topics relevant to the research (e.g. Clinical neurophysiology, pathophysiology of epilepsy, animal models of epilepsy, dynamical modeling, dynamical measures, global optimization, and seizure prediction). Teleconferencing allows direct participating by the ASU group. A new RAID system, enabling rapid access to data and programs by all investigators in each participating lab, has been installed. The group is working actively with the University of Florida Office of Technology Licensure to identify industry partners in order to effect transfer of technology. Publications resulting from the collaboration are listed below.

Completed Publications in Scientific Journals - Peer Reviewed

Iasemidis LD, Pardalos P, Sackellares JC, and Shiau DS. Quadratic binary programming and dynamical system approach to the predictability of epileptic seizures, Journal of Combinatorial Optimization 5(1):9-26, 2001.
Pardalos PM, Sackellares JC, Yatsenko VA, and Butenko SI. Nonlinear dynamical systems and adaptive filters in

biomedicine. Annals of Operations Research, in press.

Pardalos PM, Sackellares JC, Yatsenko VA, Yang MCK, Shiau DS, Chaovalitwongse W. Statistical information approaches to modeling and seizure detection in the epileptic human brain, in press.

Publications submitted to Scientific Journals - In review

Iasemidis LD, Pardalos P, Sackellares JC, and Shiau DS. Global optimization and nonlinear dynamics to investigate complex dynamical transitions: application to human epilepsy, IEEE Transaction on Biomedical Engineering, in revision.

Pardalos PM, Sackellares JC, and Yatsenko VA. Optimization and dynamical analysis of EEG data., submitted to OR Spectrum, 2002.

Pardalos PM, Sackellares JC, Carney PR, Yatsenko VA, and Srivastava A. Reconstruction of dynamical systems and nonlinear analysis in a transgenic epileptic mouse, submitted to Physica D, 2002.

Iasemidis LD, Pardalos PM, Sackellares JC, Shiau D-S, Chaovalitwongse W, Principe JC, Yang MCK, Yatsenko VA, Roper SN. Seizure warning algorithm based on spatiotemporal dynamics of intracranial EEG, submitted to Mathematical Programming, 2002.

Patents

Sackellares, JC and Iasemidis, LD. "Seizure Warning & Prediction" Patent Application. Serial Number 09/400,982 (UF - 10131).

Book Chapters

Pardalos PM, Knopov P. S., Uryasev S., and Yatsenko, V. Optimal estimation of signal parameters using bilinear observations}" In A. Rubinov, (Ed.) Optimization and Related Topics. Kluwer Academic Publishers (2000), pp. 103-117.

Iasemidis LD, Principe JC and Sackellares JC. Measurement and quantification of spatiotemporal dynamics of human epileptogenic seizures. In Akay M. (Ed) Nonlinear Signal Processing Volume II: Dynamic Analysis and Modeling, pp294-318, IEEE Press, NY, 2001.

Sackellares JC, Iasemidis LD, Pardalos, PM, Shiau DS. Combined application of global optimization and nonlinear dynamics to detect state resetting in human epilepsy. In: Biocomputing, Pardalos PM and Principe J, Eds., pp140-158, Kluwer Academic Publishers, 2002.

Iasemidis LD, Shiau DS, Pardalos PM, Sackellares JC. Phase entrainment and predictability of epileptic seizures. In: Biocomputing, Pardalos PM, Principe J, Eds, pp.59-84., Kluwer Academic Publishersq, 2002

Pardalos PM, Sackellares JC, Yatsenko V. Classical and quantum controlled lattices: self-organization, optimization and biomedical applications. In: Pardalos PM and Principe J. (Eds), Biocomputing, pp.199-224, Kluwer Academic Publishers, 2002.

Pardalos PM, Iasemidis LD, Yatsenko V, and Sackellares JC. Global optimization approaches to reconstruction of dynamical systems related to epileptic seizures. In Massalas CV et al, (Eds.), Mathematical Methods in Scattering Theory and Biomedical Technology, World Scientific, 2002, in press.

Abstracts and Preliminary Communications:

Iasemidis LD, Pardalos P, Shiau D-S, and Sackellares JC. Quadratic integer optimization and nonlinear dynamics for prediction of epileptic seizures. Proceedings of the Annual Meeting of the American Association for the Advancement of Science, p A 40, San Francisco, CA, 2001.

Iasemidis LD, Shiau D-S, Luo Q, Gilmore RL ,Roper SN, Pardalos P, and Sackellares JC. Resetting of the epileptic brain follows the occurrence of epileptic seizures. Presented at the conference on Biocomputing 2001, University of Florida, Gainesville, Florida, 2001.

Sackellares JC, Iasemidis LD, Pardalos PM, Chaovalitwongse W, Shiau DS, Roper SN, Gilmore RL, Carney PR, Principe JC: Performance characteristics of an automated seizure warning algorithm (ASWA) utilizing dynamical measures of the EEG signal and global optimization techniques. Epilepsia 42 (Suppl 7): 41, 2001.

Carney PR, Iasemidis LD, Pardalos PM, Srivastava A, Won J, Shiau DS, Lee N, MacLennan AJ, Sackellares JC:. Predictability of seizures in an epilepsy-prone transgenic Mouse Model, Epilepsia 42 (Suppl 7): 225, 2001.

ISSUES:

Progress in development of computer paradigms and data acquisition has been more rapid than expected. In the near term, we anticipate that the speed of existing computers and equipment to transfer previously recorded EEG data to DVD will be a limiting factor. We need to identify funds to update or replace outdated equipment.

PI: **SHAIN, WILLIAM**
Biggs Laboratory
Wadsworth Center
PO Box 509
Albany NY 12201-0509
T: (518) 473-3630
shain@wadsworth.org

PROJECT TITLE: Brain prostheses: Tissue compatibility & integration

PARTNERS' NAMES AND AFFILIATIONS:
James N. Turner
Biggs Laboratory, Wadsworth Center
PO Box 509
Albany NY 12201-0509

M. Isaacson
School of Applied and Engineering Physics
Cornell University, Ithaca NY

H.G. Craighead
School of Applied and Engineering Physics
Cornell University, Ithaca NY

W. M. Saltzman
Department of Chemical Engineering
Cornell University, Ithaca NY

GRANTING NIH INSTITUTE/CENTER: National Institute of Biological Imaging and Bioengineering (NIBIB)

ABSTRACT:
Nanofabricated neural prosthetic devices provide tremendous potential for furthering our understanding of central nervous system (CNS) function and treating CNS disease and injury. Such devices will permit precise localization of targets and control of electrode function. However, the success of these devices is presently limited by reactive biological responses. These experiments are designed to compare and contrast events underlying early and prolonged responses observed following prosthesis insertion in order to develop strategies for successful design and use of neural prosthetics. We will identify the signaling events that produce these responses, identify the source of these signals, and use technology advances in prosthesis insertion, prosthesis design, and pharmacological delivery, to control these events. Reactive responses will be described using our procedures to assess biochemistry and function of individual cells in complex 3-D samples. Confocal microscopy of thick sections (~ 100 Fm thick) is coupled with immunohistochemical and fluorescence in situ hybridization to permit identification of neurons, astrocytes, and microglia, measure cytokine production, activation of second messenger signaling pathways, elaboration of extracellular matrix components, and other specific cell products. These techniques permit simultaneous evaluation of changes in cell morphology and tissue distribution as well as the relative abundance of messenger RNAs or gene products. We will determine the cells participating in reactive responses, describe cell-cell and cell-prosthesis interactions, and, with our data analysis capabilities, quantify these observations. Cell and organotypic cultures, developmental staging, and mouse genetic models will be used to test experimental hypotheses developed from observations in adult rats. Prostheses

will be made using nanofabrication techniques, surfaces will be modified by chemical, biochemical, and physical (topographic) methods. Pharmacological interventions will be tested by systemic application as well as incorporating microfluidic elements into prosthetic devices. Results from these experiments will provide important new information for the intelligent design of improved biomaterials and micro-devices to control dynamic biological events in the CNS and insure the successful long-term performance of neural prosthetic devices.

STATUS OF RESEARCH AND PARTNERSHIP:

The primary focus of activities this year has been to develop and implement methods to provide micro-fabricated neural prosthetic devices with functions for release of biologically active compounds. Experiments were designed to first demonstrate the effectiveness of target compounds - anti-inflammatory agents - using peripheral injection, demonstrate the abilities to apply drugs locally in the brain to produce a similar effect, and initiate experiments to incorporate drug delivery into prosthetic devices. Dexamethasone and cyclcosporin A were administered by peripheral injection. Dexamethasone modified early and prolonged reactive responses by reducing astrocyte activation; however, it appears that during the period of early reactive responses microglia and vascular responses may be increased. Local drug administration was achieved using slow release from hydrogel (poly(ethylene-co-vinyl acetate) ribbons that were the same length as our devices, but with larger cross-sectional dimensions, 400 x400 vs 100x100 :m. Drug content of these materials produced initial tissue concentrations similar to that calculated from the peripheral injections; however, the time of drug delivery was much longer. Methods for coating devices with these materials are being developed. Biological responses to these inserted materials will be presented. Drug delivery via fluidic channels on prosthetic devices is also being developed. Methods for channel fabrication have been established and their flow properties characterized. Significant progress has also been made in establishing methods to assess responses of neurons to inserted devices. This is a particularly difficult problem because of their densities and complexity of the 3-D morphologies of these cells.

During the last year W.M. Saltzman has joined our partnership. Dr. Saltzman's expertise is in the develop and use of polymers for the release of bioactive drugs and proteins into tissues.

ISSUES:

Our biggest challenge has been establishing effective communication between groups. This is made difficult by both different geographical locations - Ithaca and Albany are about 180 miles apart and differences in vocabulary and experimental approaches inherent in the different disciplines. Regular communications is critical. We have developed the following strategies to successfully meet this challenge. Since regular commuting is unreasonable, we use a variety of methods/media for regular communication. Face-to-face meetings of the entire group are held at a location half-way between the institutions. This allows for introductions of new participants and real brain storming in the absence of interruptions that one can never seem to avoid when at home. Visits by individuals occur on a regular basis so that critical fabrication and experiments are a common experience. Tele-conferencing, and most recently video-conferencing, work well once the participants become comfortable with these media. Regular e-mail communication become a regular part of daily activities when rapid responses are needed. Using this battery of methods we do very well.

PI: **SKALAK, THOMAS C.**
Department of Biomedical Engineering
Box 800759 Health System
University of Virginia
Charlottesville, VA 2290
T: (434) 924-0270
tskalak@virginia.edu

PROJECT TITLE: Integrated Control of Vascular Pattern Formation

PARTNERS' NAMES AND AFFILIATIONS:
Gary K. Owens, Richard J. Price

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
This Bioengineering Research Partnership assembles a team led by two biomedical engineers and a molecular physiologist to focus on the integrative control of vascular pattern formation. While vascular assembly and pattern formation will be needed as critical elements of successful therapeutic collateralization of progressively ischemic organs and in tissue engineering of various tissue substitutes in the future, remarkably little is known of the cells involved, the array of signal molecules and their genetic regulation, and the biophysical factors regulating the spatial and temporal dynamics of vascular pattern formation. Key questions now are: what is the origin of cells responsible for the investment of arterioles with contractile cells and what are the signals that control their proliferation, migration, and differentiation? An integrative systems approach is proposed to measure the dynamics of arteriolar pattern formation in vivo across time scales from the embryo to the adult, and spanning spatial scales from genes to cells to whole networks, and to create a new generation of computational approaches to understand the complex interplay of multiple interacting cells and signal molecules. The specific aims are 1) to determine the role of PDGF and TGF-$ in arteriolar pattern formation during embryonic development, 2) to determine the cell types involved, role of PDGF and TGF-$ signaling, and spatial and temporal patterns of arteriolar assembly in adults, and 3) to develop and use a new cell-based computer simulation to perform integrative spatio-temporal analysis of the arterialization process in the embryo and adult, including multi-signal control of fibroblast and smooth muscle cell proliferation, migration, and differentiation. The multidisciplinary team will utilize unique gene-targeted mice in conjunction with innovative in vivo measurements, and integration of the data into the new computational models will improve understanding of the gene circuitry regulating arteriolar pattern formation. This focused partnership with three investigators who have worked together previously brings a unique set of complementary tools to bear on the problem. Year 1 milestones are to obtain the first microvessel mappings of contractile cell recruitment in transgenic mouse embryonic tissues, to implement spatial guidance of arteriolar pattern formation through application of focal growth factors in adult window chambers, and to implement a novel computational model of arterialization that represents smooth muscle cells and fibroblasts discretely. The long term goal is to define the mechanisms that control arteriolar pattern formation, and to provide the basis for powerful therapeutic vascularization procedures that function in the native environment in vivo.

STATUS OF RESEARCH AND PARTNERSHIP:
To chronically study microvascular remodeling in individual networks subjected to focally applied growth factors, we use a window chamber model. To date, our work has focused on how the spatial and temporal manipulation of focal vascular endothelial growth factor (VEGF) and Angiopoietin-1

(Ang-1) application influences morphological changes in the network architecture of subcutaneous vessels. VEGF, a multifunctional cytokine, mediates angiogenesis by promoting endothelial cell recruitment and proliferation, while Angiopoietin-1 (Ang-1) is suggested to have a role in vessel stability and perivascular cell recruitment. Alginate beads (100 mm-diameter) containing VEGF or vehicle control were inserted into opposing window quadrants of circular window chambers. After 7 days, a second alginate bead containing Ang-1 was administered into the windows. Optical images were acquired using a light microscope 1, 7, 14, and 21 days after bead implantation. Local stimulation by exogenous VEGF produces an altered network morphology that initially has an increased proportion of small vessels that persist for up to 14 days but are later pared down. The administration of exogenous Ang-1 after VEGF stimulation significantly increased the percentage of vessels with diameters less than 25 microns at Day 21 to 76±7%. This important result indicates that applying Ang-1 to a tissue that has become highly vascularized due to an initial VEGF application helps to stabilize the network in that highly vascularized configuration. This result not only supports the role of Ang-1 as a stabilizing factor for microvessel networks, it also highlights the importance of the temporal regulation of growth factor application in altering and maintaining normal network structure in guided arterialization.

We have begun characterizing vascular phenotypes in mice that have undergone genetic manipulations designed to uncover the role of selected growth factors and their receptors in mediating large vessel and microvessel remodeling in the skeletal muscle, myocardium, mesentery, and kidney. The first genetically modified mouse exhibits lacZ expression in smooth muscle cells that are expressing SM-MHC. In large arteries and microvessels, this mouse exhibits a mosaic pattern of SM-MHC expression, presumably due to temporal alterations in SM-MHC expression. A second transgenic mouse, a cross between a mouse that drives cre expression via the SM-MHC promoter and a ROSA/stop/lacZ mouse, expresses lacZ in all cells that have made SM-MHC at any point in their history. The third mouse is a single transgenic mouse in which GFP expression is driven by the CR-1 promoter. This mouse, as expected, exhibits GFP expression in all cells. In the near future, this mouse will be crossed with a second transgenic mouse (cre driven PDGF$-R dominant negative) to produce a mouse in which cells expressing the dominant negative form of the PDGF-R$ are the only cells in the tissue not expressing GFP. This mouse will be a valuable tool for studying the role of PDGFR-$ in the recruitment of smooth muscle precursor cells to developing microvessels in the adult animal.

A computational automata model has been developed and implemented to simulate the growth of new capillary networks and their subsequent arterialization. The model is comprised of multiple discrete cells of various phenotypes including endothelium, mesenchymal fibroblasts, pericytes, and smooth muscle cells. The cells proliferate, migrate, and differentiate according to a set of interaction rules defined by independently measured cell behaviors in response to the growth factors PDGF-B, TGF-beta, and VEGF. Results predict the capillary length density and arterialized portion of the new vascular networks quantitatively, and agree well with experimental findings. We believe this type of model has the potential to represent multiple types of tissue patterning events and thus offers a rational design tool for therapeutic arterialization.

The partners' laboratories have recently been moved into contiguous space in a new research building, joint lab meetings have provided for planning and discussion, and one MD/PhD student is now being jointly advised by the investigators. The partnership is working effectively.

PI: **SKLAR, LARRY A.**
Cancer Research Facility Room 219A
University of New Mexico Health Sciences Center
Albuquerque, NM 87131
T: (505) 255-7790
lsklar@salud.unm.edu

PROJECT TITLE: 7 TMR (GPCR) Drug Discovery, Microfluidics & HT Flow Cytometry

PARTNERS' NAMES AND AFFILIATIONS:
Tione Buranda, PhD, Research Asst. Professor of Pathology, UNMHSC
Bruce Edwards, PhD, Research Associate Professor of Pathology, UNMHSC
Gabriel Lopez, PhD, Associate Professor Chem. Engineering and Chemistry, UNM SOE
Eric R. Prossnitz, PhD, Associate Professor of Cell Biology and Physiology, UNMHSC
Hy D. Tran, PhD, Asst. Prof. Mechanical and Electrical Engineering, UNM SOE
Andrea Mammoli, PhD, Asst. Professor Mechanical and Electrical Engineering, UNM SOE

ABSTRACT:
High throughput (HT) screening is integral to drug discovery. While flow cytometry is known for its ability to measure cell responses, its power in the homogeneous analysis of ligand binding or molecular assembly and its potential for high throughput are not well-recognized. The possibility of displaying virtually any molecule in a format compatible with particle-based analysis as well as the novel approach of plug-flow flow cytometry for sampling times ~1 sec could make flow cytometry a powerful alternative for the real-time analysis of molecular interactions. Thus, we propose four projects that bring together expertise in bioengineering and biomaterials, receptors and cell biology, and flow cytometry instrumentation. The first two projects concern biomaterials. In the first project, we propose to express the proteins relevant to signal transduction and termination (seven trans-membrane receptors - 7TMR, receptor tails, G protein sub-units, arrestins, and receptor kinases) in forms appropriate for flow cytometry. These proteins will have epitope tags suitable for homogeneous attachment to beads as well as fluorescent groups suitable for detection by conventional flow cytometry. In the second project, we will employ biomaterial display and detection strategies compatible with flow cytometric analysis. Beads will be used as platforms to display the molecules, to analyze molecular assemblies, to examine enzymatic activities, and to examine inhibition by combinatorial drug libraries. Projects 3 and 4 will involve instrumentation development, fluidics, micro-machines, and automation. In the third project, we will develop fluid handling approaches for cells and beads. We will target throughput rates of 1 sample per second, or near the industrial standard of 100,000 samples per day, using commercial fluid handling components for the types of assays described in Projects 1 and 2. In the fourth project, we will develop and implement micro-fluidic sample handling approaches compatible with flow cytometry using novel elastomer-based micromachine technology. We have set a goal of 10 samples per second or 864,000 samples per day, exceeding the industrial throughput standard by nearly an order of magnitude. By integrating bioengineering, biomaterial, molecular, cellular and flow cytometric expertise, we expect to develop test platforms for high throughput analysis of molecular interactions with commercial potential in drug development. The resulting technological advances will allow us at the same time to define mechanistic details of cell activation through 7TMR mediated pathways.

STATUS OF RESEARCH AND PARTNERSHIP:
The entire BECON team meets monthly and the project teams meet weekly. The team has had

both biological and technological successes. In the biological arena, we have established several assays suitable for screening by high throughput flow cytometry that include: cell-based assays for GPCR and integrin ligand binding using fluorescent ligands; cell-based assays for GPCR initiated cell responses such as intracellular calcium elevation; cell-based adhesion assays for cells in suspensions; bead-based assays for GPCR molecular assemblies involving intracellular components; bead-based assays for GPCR tail peptide assemblies and phosphorylation; liposome/bead assays of transmembrane transport; and generalized bead-based approaches to analyze of protein complexes. These assays are also being used to characterize molecular interactions of normal and mutant signaling partners for basic research. In the technological arena, we have described the first generation of high throughput flow cytometry "plug flow" that was capable of 10 end point assays per min, 4 on-line mixing experiments per min, and a concentration gradient for secondary screening of a compound in about 2 min. The second-generation instrumentation (Patent Pending, HyperCytTM) approaches a rate of 100 samples/minute with <1% particle carryover from well to well. This approach uses air bubbles to separate ml sized samples with low carryover. We have developed on-line microfluidic mixing for submicroliter samples and sampling rates up to 20 samples per minute. We are now in the process of coupling HyperCyt to high speed sorting. Taken together, these approaches provide new opportunities for low cost, small volume, high throughput screening of cell and bead-based molecular target assays at rates approaching 100,000 assays per day. Combined with multiplexed assays for genomic and proteomic screening, they promise to make flow cytometry a competitive screening tool in research and commercial settings.

ISSUES:
None

PI: **SMITH, WILLIAM A. , D.ENG., P.E.**
Department of Biomedical Engineering / ND20
The Cleveland Clinic Foundation
9500 Euclid Avenue
Cleveland, OH 44195
T: (216) 445-9334
wasmith@bme.ri.ccf.org

PROJECT TITLE: MagScrew TAH Testing thru Preclinical Readiness

PARTNERS' NAMES AND AFFILIATIONS:

The Cleveland Clinic Foundation
Wilson Greatbach, Ltd.
Foster-Miller Technologies, Inc.
Whalen Biomedical, Inc.

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

ABSTRACT:
The fundamental goal of the proposed program is to bring to the point of clinical readiness a new, electrically powered, totally implantable TAH, based on the MagScrew actuator and the biolized blood pump. The specific aims to meet this goal are: (1) To design and develop an advanced technology, fail safe, electronic control unit (ECU), which will maintain the patient's life after an electrical failure, until maintenance is performed. The ECU also contains hardware and patient monitoring capability, and a telemetry function. (2) To build and test refined versions of the remaining system components, based on current state of the art technology. (3) To integrate the components into a functional, complete system. (4) To perform in-vivo performance tests, exercising system capabilities. (5) To perform in-vivo durability tests. (6) To perform bench endurance tests. (7) To complete this work in compliance with FDA Design Controls Regulations.

As a consequence of this design and testing effort, surgeons will have another, superior choice among relatively limited TAH alternatives. The "biolized" pump of the MagScrew TAH has pericardial valves combined with biological, protein blood contacting surfaces, and a long track record of extremely rare thrombo-embolic episodes in calves, despite the absence of anti-coagulation. In addition, the MagScrew actuator is the conceptually simplest and most rugged of those available for TAHs, with very few contacting or rubbing surfaces. Mechanical failures have very few possible sources, which clearly increases both reliability and long-term durability. The "fail safe" controller will address the residual pinched wire, corroded solder joint, software hang-up and similar problems that are unavoidable, even with the best fundamental design, and rigorous quality control, in sophisticated, densely packed electronics that are implanted in a hostile environment, and that have caused failures of other, older systems. While the clinical need for TAHs is consistently estimated to be much smaller than that for VADs, it is of a size both nationally and internationally to be of commercial significance. In the United States, it may exceed $1B per year in potential sales. The TAH market will support several suppliers, if not as many as now pursuing the VAD market. To those patients who will need a TAH, the potentially very limited supply of alternatives is of literally life and death significance.

STATUS OF RESEARCH AND PARTNERSHIP:
Work is well under way. The majority of effort to date has involved design of hardware. Over the next few weeks, components will be released to manufacture for the new actuator and blood pump. The electronic control unit is being breadboarded and de-bugged in functional units and making good progress. A considerable effort is being expended on management systems to assure that a first-class Design History is assembled. In general, the technical work is on track.

The partnership is working reasonably well, with each group contributing its technical expertise. The formal, legal contracting among the groups is moving a little more slowly. In that a commercializable product is involved, many "i's" have to be dotted and "t's" crossed; no fundamental issues exist.

ISSUES:

Time is perhaps our biggest issue. The first contract year ended up being less than 12 months, for administrative issues, but the research still requires the allocated time. There is a constant dynamic between making a step as good as it can be and calling a task done, so that the program can move on.

Communication is also a factor that must always be considered. The system design is tightly integrated, and it is very difficult for any one partner to make a decision without information from another partner. We have to watch this area every day.

PI: **SNYDER, ALAN J.R, PH.D.**
Departments of Surgery and Bioengineering
500 University Drive, C4864/H151
Hershey, PA 17033
T: (717) 531-7068
asnyder@psu.edu

PROJECT TITLE: Biomedical applications of electroactive polymers

PARTNERS' NAMES AND AFFILIATIONS:

Qiming Zhang, PhD.
Materials Research Laboratory
The Pennsylvania State University
University Park, PA

Mary I. Frecker, PhD.
Dept. of Mechanical Engineering
The Pennsylvania State University
University Park, PA

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
The objective of the Bioengineering Research Partnership program is to refine materials and establish methods for application of electroactive polymers in prosthetics and interventional medical devices. The electroactive materials of interest to us are those that undergo substantial shape change when exposed to an electric field. They are attractive as actuators because of their high energy density - the amount of energy that can be imparted to a load for a given volume or mass of active material, the magnitude of the strain response to an applied field, and their flexibility and toughness when compared with more common electroactive ceramics. Both "found" materials and materials developed expressly for electromechanical activity have been shown exhibit strains of 5% or more and elastic energy densities of one Joule per cc or more.

Two target application areas have been chosen: (1) next-generation prosthetic blood pumps for treatment of end-stage heart disease, and (2) robotic manipulators for minimally invasive surgery, particularly for use in confined spaces such as the thorax. These disparate applications share the need for very compact, efficient and uncomplicated means of actuation. Both suffer today from the need for bulky actuation mechanisms that must remain physically distinct from the parts which pump blood or manipulate tissue. The technology to be developed under this program will blur the lines between structure and actuator, leading to modes of therapy that are not currently available.

The Materials Research partner is working to optimize electroactive polymers for use in the target medical devices, and develop methods for fabrication of the required multilaminate actuators. As these materials are fundamentally different from the active materials of actuating mechanisms used by engineers in the past, the Mechanical Engineering partner is working to develop new design methodologies. The Bioengineering partner is developing prototype devices to demonstrate the potential of the technology and lay the ground work for full development of new devices. Device development is staged so that simpler, proof-of-concept designs are built first, followed by more sophisticated designs as materials and design tools are developed.

STATUS OF RESEARCH AND PARTNERSHIP:
Materials development work over the first 18 months of the project has focused, as planned, upon optimal processing of vinylidene fluoride-trifluoroethylene copolymers p(VDF-TrFE) for use as actuators, and upon development of new high-performance materials. Linear actuators fabricated from rolled films are routinely fabricated. Present efforts are focused upon improvements in reliability

and processing of biaxially-active films for pump applications. The maximum work that can be extracted from an electroactive material is directly proportional to its dielectric constant, and efforts are underway to develop high dielectric constant polymers using oligomer fillers. Thus far, composite materials have demonstrated dielectric constants over 200 have been demonstrated in composites with favorable mechanical properties. Continued work is focused upon improvements in oligomer distribution and particle size, and in quality of cast films. The materials research partner has also built prototype electroactive polymer diaphragm micropumps that show promise of having superior volumetric efficiency compared with solid state devices using piezoceramics.

The mechanical engineering partner has focused upon development of both analytical and finite element models. An analytical model of a three-element unimorph-type steerable end-effector with kinematics that mimic the index finger was developed and both deflection and force generation was compared with prototypes. The model is being refined to account for electrode and bonding layers. An analytical model for a multilayer membrane actuator for blood pump application is currently being developed. The basic governing equations for a three dimensional active shell using the electrostrictive P(VDF-TrFE) copolymer have been derived. The model will be used to predict strain and swept volume as a function of the applied electric field. Shells of various layer configurations and of various shapes from flat to hemispherical can be handled. Ongoing work includes implementing a numerical method to solve the governing equations and to serve as the basis for thickness, layup and shape optimization.

The bioengineering partner has concentrated upon testing of finished materials, development of proof-of-concept prototypes and investigation of different forms of actuators that will take best advantage of material properties and processing requirements. We have demonstrated movement of a one degree-of-freedom tool tip using a thin strip actuator, and have made progress in developing mathematical models for the actuators that can be used in deriving movement control systems. We have also demonstrated electrically induced volume displacement in single-layer and thin multilaminate diaphragms and are evaluating relative performance with alternative materials and fabrication techniques. Mainly through finite element simulations, we are also investigating the use of non-planar (tubular) structures that would offer advantages in electrode configuration and would be scalable to micron sizes.

ISSUES:
The partnership is operating effectively. Whole-group meetings, one-on-one meetings and electronic communications among partners are all quite effective; we rely most upon electronic communications. Effective partners are motivated chiefly by the desire to work on new problems in a collaborative area. Joint funding enables them to devote the necessary time to the work.

PI: **SOPER, STEVEN A.**
Department of Chemistry
232 Choppin Hall
Louisiana State University
Baton Rouge, LA 70803-1804
T: (225) 578-1527
F:(225) 578-3458
chsope@lsu.edu

PROJECT TITLE: Micro-Instrument Platforms for Genetic-Based Assays

PARTNERS' NAMES AND AFFILIATIONS:
Robin L. McCarley (Chemistry, LSU)
Robert P. Hammer (Chemistry, LSU)
Michael C. Murphy (Mechanical Engineering, LSU)
Wanjun Wang (Mechanical Engineering, LSU)
Dimistris Nikitipoulos (Mechanical Engineering, LSU)
Kevin Kelly (Mechanical Engineering, LSU)
Jost Goettert (CAMD, LSU)
Francis Barany (Molecular Genetics, Cornell Medical College)

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

ABSTRACT:
The goal of this research effort is to bring together a multi-disciplinary team (Chemists, Engineers, Life Scientists) to develop integrated microfabricated tools to carry out PCR/LDR (Polymerase Chain Reaction/Ligase Detection Reaction) assays for the detection of low abundant cancer diagnostic markers (K-ras mutations, 19 single base mutations). The devices to be fabricated as part of this research program are:

- Fast thermal cyclers for PCR amplification of DNAs in nanoliter volume chambers with automated sample and reagent delivery.
- Micro-electrophoresis devices machined in polymethylmethacrylate (PMMA) containing multiple separation channels for the high speed processing of ligation products.
- DNA hybridization chips (micro-arrays) fabricated in plastics with nano-fluidic channels for LDR capture. The DNA micro-array substrates will offer robust immobilization chemistries that can tolerate typical thermal and chemical DNA hybridization/denaturation conditions.
- Ultrasensitive near-IR fluorescence instrumentation using solid-state components (diode lasers and detectors), which can be operated in a scanning mode to read multiple electrophoresis channels and/or DNA micro-arrays with extremely high sensitivity.
- Ultra-bright near-IR fluorescent probes appropriate for use with miniaturized near-IR detectors and for DNA micro-array readout. These probes will be configured in a two-color, four-lifetime format, which will allow the simultaneous readout of 8 unique probes.
- Injection molding of miniaturized plastic devices for maximizing production rates and minimizing fabrication costs.
- Materials characterization of the unique substrates that will be used for the micro-electrophoresis and hybridization-based assays. Also, new bonding procedures will be investigated to aid in device assembly.
- Micro-pumps for nanoliter per minute volume flow rates in microchannels used for fluid handling in pressure driven systems.

STATUS OF RESEARCH AND PARTNERSHIP:
We are in the second year of our project and have recently completed our second annual report to the NIH. As a matter of fact, we have published (or in press) 15 papers that have been supported by funds from this grant and are currently writing 2 patent applications. In terms of personnel, we are supporting the work of 3 post-doctoral associates, 5 Ph.D. students, 3 masters students and 3 undergraduate students with funds derived from this grant.

ISSUES:
There are no technical or logistical issues associated with our BRP project.

PI: **STEPHANOPOULOS, GREGORY**
Department of Chemical Engineering,
Massachusetts Institute of Technology
Room 56-469
Cambridge, MA 02139
T: (617) 253-4583
gregstep@mit.edu

PROJECT TITLE: Linking Genomics to Function via Metabolic Phenotyping

PARTNERS' NAMES AND AFFILIATIONS:
Joanne K. Kelleher, George Washington University Medical Center
Steven R. Gullans, Brigham's and Women's Hospital, Harvard Institute of Medicine

GRANTING NIH INSTITUTE/CENTER: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

ABSTRACT:
"Metabolic Phenotyping" is the process and methods employed to describe cell and tissue physiology using intracellular fluxes as determinants of the cellular metabolic state. Combined with transcription data, the metabolic phenotype provides a rich framework for linking genome to function. This partnership integrates the expertise of metabolic engineers, metabolic physiologists and clinical investigators to discover new relationships relevant to diabetes and obesity. Recombinant strains and transgenic animal models are often found to exhibit small functional differences despite specific changes at the genetic level while, in other cases, single gene alterations result in profound phenotypic variations. Although a first step in explaining such macroscopic differences is to probe the full detail of the expression phenotype by genome-wide expression measurements, transcription data alone are insufficient to elucidate the actual metabolic state of a cell and its functions.
The latter require information about intracellular metabolic fluxes, which constitute fundamental determinants of cell physiology and excellent metrics of cell function. Determination of intracellular fluxes follows a systems approach termed metabolic reconstruction, whereby the entire metabolic network is configured such as to best represent macroscopic rate data and isotopic label distribution measurements. Isotopic studies are carried out with a variety of tracer techniques. Carbon fluxes are estimated using both 13C and 14C labeled precursors. Additional insight is provided by release of 3H label from metabolites as 3H2O. To ensure optimal use of isotopic tracer labeling and bioreaction network representation, issues of observability, redundancy, and solution stability must be adequately addressed. Along with flux determination, metabolic reconstruction validates the structure of metabolic networks and helps determine points of flux control that define targets for further genetic manipulation.

Differential transcription data are obtained by DNA microarrays for mouse genes involved in central carbon metabolic, gluconeogenic and lipid biosynthetic pathways. Additionally, other genes will be identified with particular expression variability under the conditions studied. We are particularly interested in identifying discriminatory genes and gene expression patterns. Bioinformatics methods and programs, developed over the past 12 years will be deployed for this purpose.
However valuable they are, transcription data and flux measurements alone have limited capacity in elucidating the complex relationship between gene activation and cell function. To achieve this, one must collect representative data from both the expression and metabolic phenotype and then find meaningful correlational structures between the two data sets. This is the general goal of this research: Identify relationships between the metabolic phenotype as defined above and

the transcriptional state as defined by expression data of consequence in pathways important to diabetes and obesity. Specific aims will focus on flux quantification in mouse hepatoma and hepatocyte cultures to elucidate glutamine metabolism and lipogenesis, other central metabolic pathways and cholesterol synthesis, the effect of nutrients, hormones and drugs including Metformin and finally, pleiotrophic effects generated by altering the normal expression of a single gene in cell models as well as in vivo animal models. The broader contribution of this research is to extend the paradigm of holistic transcriptional investigation introduced by DNA microarray technologies to the study of metabolic level processes by metabolic phenotyping. As such, it holds the promise of identifying points in metabolism affected by the action of drugs or genetic modifications thus guiding future programs of drug development and gene therapy.

STATUS OF RESEARCH AND PARTNERSHIP:

A major focus of the past year's work has been the development and validation of a 13,000 gene mouse microarray using oligonucleotide probes. Oligonucleotide probes were obtained from Operon and spotted in triplicate on each slide yielding 39,000 spots per array. A first objective was to determine the array to array variation. Using five identical arrays to assess mouse hepatoma gene expression, we estimated the mean coefficient of variation for both within-array measurements, and for all the measurements from all of our arrays. The mean CV for the triplicate within-array measurements was in the range of 0.13-0.21. When we combined the measurements from all of our arrays, the mean CV was 0.20. This low value of the CV means that combining all sources of variation (array hybridization, reverse transcription, experimental variation), we can expect the observed ratio for any gene to differ by about 20%. In terms of fold analysis, this means that ratios which are greater than 1.4 and less than 0.6 may be considered to be differentially expressed at a 95% confidence limit. The second objective was to ascertain differential expression in our arrays. Mouse muscle and testes RNA were hybridized against each other to determine differential expression. After normalization to a mean intensity ratio of 1.00, each array was examined to determine the number of spots beyond a 2-fold change in ratio, which is the normal accepted standard in the field. From our analysis, we found that 4.5% of the spots were beyond the 2-fold limits in the control (muscle vs. muscle) array. For the arrays comparing muscle RNA with testes RNA samples, 13-21% of the spots were beyond the 2-fold change limits. This means that we observe about 3-4 times as many differentially expressed spots in our experiments as compared to the control, thus ensuring that our microarrays can detect differential expression.

Besides the mouse microarray development and validation, we have also developed a number of metabolic markers that will be employed as flux indicators in the metabolic phenotype data set. We are presently developing a new MS-based method for acquiring metabolic data in a high throughput fashion. Finally, we are developing methods for discovering the correlational structures among the two data sets, as well as methods for discovering characteristic patterns within and among such data. Powerful pattern discovery methods are applied for this purpose in collaboration with the pattern discovery and data mining group of IBM. Regarding the Partnership our work to date utilizes the expertise of the three Partners and involves interactions utilizing these skills. Dr. Kelleher is primarily responsible for coordinating mammalian metabolic flux studies. Dr. Gullans is focused on the development and validation of the cDNA microarray technology. Dr. Stephanopoulos is responsible for bioinformatics and integration of metabolic and flux data. We have developed an additional collaboration with Chris Mantzoros, MD, Beth Israel Deaconess Medical Center. Dr Mantzoros brings to our partnership expertise in mouse models of obesity and diabetes as well as clinical expertise in human metabolic diseases. The collaboration with Dr. Mantzoros has allowed us to include mouse models at an early stage of our study and to explore the unique issues linking function to the in vivo metabolic phenotype.

PI: **THIEL, PATRICIA A.**
Deptartment of Chemistry
Iowa State University, Ames, IA 50011
Ph: 515-294-7871
Thiel@ameslab.gov

Co-PI: Svetlana A. Shabalovskaya
Institute for Physical Research and Technology, Ames, Iowa 50011
T: (515) 294-1293
shabalov@ameslab.gov

PROJECT TITLE: Design of biocompatible NiTi surfaces

PARTNERS' NAMES AND AFFILIATIONS:

Dr. Gianni Rondelli, CNR-TEMPE, Institute for Material Technology and Energetic Processes, Milano, Italy.
Professor John Wataha, Medical College of Georgia School of Dentistry, Augusta, US.
Dr. Parker, School of Medical Sciences, University of Nottingham Medical School, Nottingham, England.

Collaborators:

Dr. L. Mikhailova, The Karpov Institute of Physical Chemistry, Corrosion and Electrochemistry Dept., Moscow, Russia.
Professor V. Itin, Tomsk Scientific Center of Siberian Branch of Russian Academy of Science, Tomsk, Russia.

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
The future of Nitinol as a biomaterial crucially depends on its surface characteristics. If a possible problem with Ni release from Nitinol implants could be solved through the design of a stable and inert surface, Nitinol would be superior to every other metallic biomaterial available at least through the first century of the millennium. Efforts to modify the Nitinol surfaces using artificial coatings, laser and plasma treatments or ion implantation have not succeed so far. A more promising direction to pursue, in the search of biocompatible surfaces, is chemical and elctrochemical modification of native NiTi, to produce surface layers that do not crack and peel during shape recovery of a device / implant. Therefore we propose:

1) To design biocompatible, highly corrosion resistant NiTi surfaces employing simple, cost-effective chemical and electrochemical procedures.
2) To use X-ray Photoelectron Spectroscopy combined with Scanning Ion Mass and Point Auger Electron Spectroscopy, and Back Scattering Electron as well as Scanning Electron Microscopy to provide extensive scientific information and understanding of Nitinol surfaces resulting after chemical, heat treatments and sterilization.
3) To use standard ASTM potentiodynamic and potentiostatic corrosion tests as well as the immersion test employing Inductively Coupled Plasma Analysis to evaluate the stability of designed surfaces and Ni release in biological media.
4) To preliminary evaluate the biocompatibility of Nitinol surfaces by exploring blood compatibility {platelet spreading, protein adsorption, cell proliferation (peripheral blood leukocytes, THP-1 monocytes)], and inflammatory mediators (expression of interlukin-1b and tumor necrosis factors - a that determine implantation outcome.

STATUS OF RESEARCH AND PARTNERSHIP:
NiTi material has been obtained from the Memry Inc. (project sponsor) and undergoing machining and processing into the samples for electrochemical and biological tests.

Experiments on chemical passivation of NiTi surfaces have been completed. Most of the planned surface analysis has been conducted on both stent wires and as-cast alloy.

Experimentation on electrochemical surface treatment is in progress.

Studies on the effect of the autoclaving and ethylene oxide sterilization protocols on NiTi surface conditions are partially completed.

Very productive collaboration with the partners of the first stage of the project (Italy, Russia).

Still in search of a qualified postdoc or visiting scientist.

Articles submitted for publication:

1. Bioperformance of Nitinol. Part I: Surface tendencies (invited article for the Int'l conference on Shape Memory and Superelastic Technologies).

2. Surface conditions of Nitinol wires, tubing and as-cast alloys. The effect of chemical etching, aging in water and heat treatment.

Articles in preparation:

3. The effect of chemical etching and aging in water on corrosion resistance of Nitinol wires and tubing with black oxide induced by manufacturing.

4. Comparative studies of corrosion resistance of Nitinol wires and tubing in as -received and heat treated states.

5. The effect of ethylene oxide sterilization on the surface conditions and biological performance of Nitinol.

ISSUES:

1. Underestimated budget.
2. Difficulties with obtaining visa for foreign scientists to work on the project. No candidates of American origin in electrochemical field.

PI: **VINCE, GEOFFREY D.**
Department of Biomedical Engineering/ND20
The Cleveland Clinic Foundation
9500 Euclid Avenue
Cleveland, OH 44195
T: (216) 444-1211
vince@bme.ri.ccf.org

PROJECT TITLE: High Frequency Nonlinear Acoustic Intravascular Imaging

PARTNERS' NAMES AND AFFILIATIONS:
Aaron Fleischman, PhD; Shuvo Roy, PhD; Nickolay Kharin, PhD (all BME, CCF)
Murat Tuzcu, MD; James Thomas, MD (all Cardiology, CCF)
Lawerence Katz, PhD - BME at Case Western Reserve University
Dov Hazony, PhD - Electrical Engoneering & Computer Sciences at Case Western Reserve University
William Tobocman, PhD - Physics at Case Western Reserve University

GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

ABSTRACT:
Intravascular ultrasound (IVUS) imaging is a technology that permits tomographic visualization of a cross section through the vessel wall. Its development has provided a powerful new method to assess plaque morphology in vivo. However, while new catheter designs are markedly improved on their predecessors, image quality has not seen significant gains due to the primitive nature of the ultrasound transducer designs. Increasing the frequency of the transducer above the current state?of?the?art 40MHz holds the potential to improve image quality, although higher frequencies are attenuated rapidly in biological media and the depth of penetration is therefore reduced. One possible method of enhancing the quality of IVUS images may be to exploit the effect of nonlinear propagation (harmonic imaging) of the ultrasound signal as it passes through the tissue. Despite the fact that harmonic imaging is now becoming a standard modality in the latest commercial B?mode ultrasound scanners with a frequency range up to 4.0 MHz, there is no evidence of attempts to develop a harmonic imaging system for significantly higher frequencies, which would be suitable for intravascular applications. In this application we propose to investigate the generation of tissue harmonics at high fundamental frequencies (20 ? 40MHz) suitable for intravascular application. This will be pioneering work in the field of medical acoustics. The major driving force for our project will be clinical necessity. We envisage that the implementation of high frequency harmonic imaging will dramatically improve image quality and allow better delineation of plaque geometry and composition. High frequency ultrasound transducers will be designed and built comprising traditional ceramic materials and novel polymeric devices fabricated using MEMS technology. Finally advanced signal processing methods will be designed and developed to accurately predict plaque composition from high frequency nonlinear acoustic data.

STATUS OF RESEARCH AND PARTNERSHIP:
Since the initiation of our award (01/01/02), we have concentrated our efforts on obtaining the personnel, equipment, and supplies to begin the project. At this very early stage we have started fabricating high frequency PZT transducers for our initial experiments and developing code for the analysis of RF data.

The development of the PVDF MEMS transducers is progressing well and we envisage characterising and testing these devices over the next few months.

ISSUES:
We are having difficulty in locating a commercially available 100MHz bandwidth needle hydrophone required for characterising our transducers. We may be required to build one "in-house".

PI: **VO-DINH, TUAN**
Oak Ridge National Laboratory
P.O. Box 2008
Oak Ridge, TN 37831-6101
T: (865) 574 6249
vodinht@ornl.gov

PROJECT TITLE: Advanced Multi-Spectral Imaging (MSI) System for Medical Diagnostics

PARTNERS' NAMES AND AFFILIATIONS:
Thompson Cancer Survival Center, Knoxville, TN 37916 (M.Panjehpour and B.F. Overholt)
College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37916 (R. DeNovo)

GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

ABSTRACT:
This project will develop a novel multi-spectral imaging (MSI) system using the synchronous luminescence (SL) concept to rapidly detect cancer in-vivo. The proposal will address the problem of real-time in-vivo identification and characterization of malignant and pre-malignant tissues in the upper gastro-intestinal (GI) tract. While presence of Barrett's mucosa is simple to detect endoscopically, at the present time dysplasia and early cancer is found only by extensive biopsies. The typical protocol is four quadrant biopsies at 2-cm intervals of the Barrett's mucosa. While this is the standard technique, it only provides 3-5 % sampling of the mucosal surface where dysplasia and diffuse cancer may be found. The remaining 97-95% of the mucosa is not sampled.

Laser-induced fluorescence (LIF) spectroscopy has already been used to detect cancer and high-grade dysplasia in Barrett's esophagus. However, that system uses a contact technique, which samples a 1 mm area of tissue at each measurement. While the contact LIF system is better than the pinch biopsy technique, a new system is needed to allow examination of the entire surface of the mucosa. To address this important need in imaging, we will develop a real time synchronous imaging system based on state-of-the-art acousto-optic tunable filter (AOTF) technology coupled to an endoscope.

A unique MSI technology using the SL technique will be developed at to obtain spatially resolved images of the slight differences in luminescent properties of malignant versus non-malignant tumors. This will provide a faster and more accurate in-vivo analysis without biopsy. The unique imaging aspect of this MSI system will provide real-time spatial information, allowing for comprehensive diagnosis of large areas of interest.

An interdisciplinary approach will be used to perform the proposed research to provide results in an efficient and cost effective manner. We will be working in close collaboration with the University of Tennessee (UT) School of Veterinary Medicine, and medical researchers with expertise in clinical studies at the Thompson Cancer Survival Center (TCSC). Following development of this technology, initial studies will be performed on two model systems, biopsied tissues as well as laboratory animals at Oak Ridge National Laboratory and UT. Once the system has been optimized, clinical in-vivo studies will be performed on human subjects at the TCSC in Knoxville, Tennessee.

STATUS OF RESEARCH AND PARTNERSHIP:
Since the initiation of this project in September of 2001, we have made significant progress in the development of a laboratory scale multispectral imaging system. This system employs a small optical parametric oscillator (OPO) laser for excitation, a fiber optic for excitation of the sample through an endoscope, an endoscope for signal collection, an acousto-optic tunable filter (AOTF) for wavelength discrimination, and an intensified charge-coupled device (ICCD) for detection. By using an OPO, we are capable of tuning our excitation light over the spectral range of 405 - 700 nm. This will allow us to not only perform reflection measurements on a sample, but also tune to the absorption wavelength of the various fluorophors in tissue. Once the appropriate wavelength is chosen, the laser is launched into a fused silica optical fiber. This fiber is then fed through the biopsy channel of a conventional endoscope to the sample. The light transmitted through the fiber then excites the sample. After excitation of the sample, the resulting scattered light is collected by the imaging bundle in the fiberscope (endoscope), and transmitted back to the AOTF for spectral separation. The particular AOTF that is currently being used in the instrument has a spectral resolution of approximately 3 nm. This allows relatively narrow spectral features to be distinguished from one another, while providing a broad enough measurement to obtain a signal sufficiently intense to measure in a short period of time. As the reflected light is passed through the AOTF, a single wavelength of light is diffracted off at a six-degree angle and imaged onto the ICCD. By using an AOTF coupled with an ICCD as the detector, it is possible to obtain images of the sample as opposed to a point-by-point type of measurement. In addition, using the ICCD allows the signal to be gated. This gating means that measurements can be performed under ambient light conditions without suffering from any background signals of significant intensity. By constructing this system, we have developed a laboratory instrument capable of providing any type of imaging format possible (reflection imaging, fluorescence imaging, synchronous fluorescence imaging, etc.).

In addition to designing and constructing the system, we have also begun its calibration and evaluation. In order to calibrate the system, as well as determine the spectral resolution of the AOTF, both a low-pressure xenon and a low-pressure neon lamp were imaged onto the ICCD at various wavelengths corresponding to atomic emission lines of these elements. After performing these scans, an algorithm was developed to calibrate the rf signal applied to the AOTF for tuning to the specific wavelength of light that was being emitted. Additionally, by scanning the newly calibrated instrument over an individual emission line of the lamps, it was possible to determine the resolution of the AOTF based upon the bandwidth of the resulting signals. Finally, we have also begun to perform analyses using this system in its reflection as well as fluorescence imaging modes to monitor the skin of nude mice.

We are working closely with our partners, the Thomson Cancer Survival Center (TCSC) and the University of Tennessee- Knoxville (UTK) in this project. We have prepared and submitted the animal study protocol to the UTK College of Veterinary Medicine. We have developed a clinical protocol for future clinical studies and had the protocol approved by the Internal Review Boards (IRBs) at both institutions (TCSC, ORNL).

ISSUES:
N/A.We are currently proceeding well in the system construction phase of the project.

PI: **WEISS, SHIMON**
Department of Chemistry and Biochemistry, Department of Physiology
University of California at Los Angeles
P.O. Box 951569, 607 Charles E. Young Drive East
Los Angeles, CA 90095-1569
T: (310) 794-0093
F: (210) 267-4672
sweiss@chem.ucla.edu

PROJECT TITLE: Development of Q-dots as biological probes

PARTNERS' NAMES AND AFFILIATIONS:

Prof. Paul A. Alivisatos, Co-PI
Department of Chemistry
University of California at Berkeley
Berkeley, CA 94720
T: 510-643-7371
F: 510-642-6911
alivis@uclink4.berkeley.edu

Prof. Ehud Isacoff
Department of Molecular and Cell Biology, 279 LSA
University of California at Berkeley
Berkeley, CA 94720-3200
T: 510-642-9853
eisacoff@socrates.berkeley.edu

Prof. Terry Machen
Department of Molecular and Cell Biology, 231 LSA
University of California at Berkeley
Berkeley, CA 94720-3200
T: 510-642-2983
F: 510-643-6791
machen@socrates.berkeley.edu

Prof. Hsiao-Ping Moore
Department of Molecular and Cell Biology, 571 LSA
University of California at Berkeley
Berkeley, CA 94720-3200
T: 510-643-6528
F: 510-643-8708
hpmoore@uclink4.berkeley.edu

Prof. Zacheus Cande
Department of Molecular and Cell Biology, 341 LSA
University of California at Berkeley
Berkeley, CA 94720-3200
T: 510-642-1669
F: 510-643-6791
zcande@uclink4.berkeley.edu

Prof. Rebecca Heald
Department of Molecular and Cell Biology, 311 LSA
University of California at Berkeley
Berkeley, CA 94720-3200
T: 510-643-5493
F: 510-643-5002
heald@socrates.berkeley.edu

Prof. Matt Welch
Department of Molecular and Cell Biology
University of California at Berkeley
Berkeley, CA 94720-3200
welch@uclink4.berkeley.edu

GRANTING NIH INSTITUTE/CENTER: National Center for Research Resources (NCRR - lead), National Institute of General Medical Sciences (NIGMS), National Cancer Institute (NCI)

ABSTRACT:
The long-term goal of this Bioengineering Research Partnerships is to develop semiconductor nanocrystals fluorescent probes (Q-dots) technology that will provide biomedical research with better tools for diagnosis of diseases and biomedical techniques and instrumentation necessary for basic research of cellular and molecular structure and fundamental life processes. This includes Q-dot probe synthesis, bio-conjugation techniques, dedicated optical instrumentation and unique imaging methodologies. We will develop optimized protocols for Q-dot synthesis with desired optical, physical and chemical properties. Various spectroscopic and structural measurements will be used to fully characterize Q-dots. This information will be fed back into the synthesis for optimization of the desired properties. Bio-conjugation schemes and labeling protocols will be developed for biomolecules in fixed and living cells.

The utility and the new possibilities opened-up by Q-dot technology will be demonstrated by studying protein trafficking and assembly in living cells and by physically mapping genes. The movements of secretory granule membranes during recycling will be tracked in living cells. Actin-based locomotion and mitotic spindle assembly will be imaged in real-time in cell-extracts. Molecular mechanism of synaptic transmitter release will be studied by following vesicle dynamics and protein trafficking in the synaptic apparatus. We will also physically map large number of distinct markers on chromosomes and combed DNA molecules and monitor the kinetics of chromosome pairing during meiotic prophase. All these demonstrations rely on the unique photophysical properties of q-dots, enabling new experiments and measurements to be performed and significant new biology to be revealed.

STATUS OF RESEARCH AND PARTNERSHIP:

Photophysics:

Extensive photophysical studies based on fluorescence correlation spectroscopy, single molecule imaging and spectroscopy, simultaneous recording of photon pair correlations (antibunching) and time correlated single photon counting (fluorescence lifetime) reveal inhomogeneity in fluorescence properties of Q-dots. It was found that ensemble quantum yield measurements are misleading and that a large subpopulation of Q-dots is initially dark. We are exploring modifications to the synthesis to reduce this phenomenon.
Colloidal quantum rods were developed that exhibit linearly polarized emission, ideally suited for orientation-sensitive applications. There polarized emission was studied by single molecule polarization spectroscopy. Such labels can be used to study conformational change in biological macromolecules.

Bioconjugation:

A new method for modulating the surface chemistry properties of Q-dots using peptides as an organic coat was developed. It provides water solubility and bio-activity by means of a recognition peptide in a one step reaction. The peptide gives the particles protein-like properties. Based on this technology, we hope to develop targeting schemes for live cell experiments.

Biological Experiments:

A cell motility assay was developed based on the endocytotic uptake of Q-dots by live cells. nanocrystals probes are more photochemically robust than organic dyes (which fade quickly) and do not perturb the cells. The ability to examine these behaviors in live cells over extended time periods, and to quantify changes in response to various molecular manipulations, provides a new tool for studying the processes of cell motility and migration.

ISSUES:

The PI joined UCLA (Chemistry & Biochemistry, Physiology) and the California Nanosystems Institute (CNSI, www.cnsi.ucla.edu). Lab renovations at UCLA are ongoing; the transition from LBNL to UCLA somewhat slowed the research. It is anticipated that the PI's group will move to UCLA in mid-April. The new labs are very well equipped and optimized for the proposed work. It is expected that by summer of 2002 the research will commence to its full scale. The transition to UCLA also generated new collaborations and applications of Q-dots technology. As a result, restructuring of partnerships might be considered.

PI: **WESTENSKOW, DWAYNE, PHD**
Department of Anesthesiology/Bioengineering
University of Utah
Salt Lake City, UT 84132
T:(801) 581-2478
drw@cc.utah.edu

PROJECT TITLE: Data Display to Detect-Diagnose-Treat Critical Events

PARTNERS' NAMES AND AFFILIATIONS:

Julio Bermudez, PhD, Dept Architecture
Joseph Orr, PhD Dept Bioengineering
David Strayer, PhD, Dept Psychology
Stefano Foresti, PhD, Dept High Performance Computing

GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

ABSTRACT
This project seeks to develop new displays for visually representing physiologic variables, to enhance a clinician's ability to see and rapidly respond to critical events. The display is to provide a comprehensive view of the surgical patient's physiologic state. The physiologic icons in the display will be organized to enhance the vision of the interrelationship between organ system functions. The display will highlight input/output relationships, by showing drug concentrations and accompanying physiologic changes. The anesthesiologist "sees" the patient on the operating table through the monitoring display. The proposed research will identify the type of display, which best helps the anesthesiologists to rapidly detect physiologic changes, to make accurate diagnostic decisions and to efficiently treat critical events.

STATUS OF RESEARCH AND PARTNERSHIP:
Last year the Bioengineering Research Partnership developed the intravenous drug display shown in Figure 1. It presents the anesthetic drugs that have been administered to the patient, shows predicted drug effect-site concentrations and predicts interactions between drugs.

The drug display was evaluated in a high fidelity simulation to determine whether this technology supports anesthesiologists when administering total intravenous anesthesia during a complex simulated surgery. Twenty-four attending (12) and resident (12) anesthesiologists, with a range of clinical experience, participated in the study. Equal numbers of attending and resident anesthesiologists were assigned randomly to two groups. The experimental group used the drug display plus traditional monitors, while the other group was assigned to the control condition (traditional monitoring only). Participants were instructed how to use the METI patient simulator (METI, Sarasota, FL) and the intravenous drug delivery devices: the DocuJect (DocuSys, Mobile, AL) bar-coded intravenous syringe drug reader and two Medfusion 3010a (Medex, Duluth, GA) pumps. Subjects induced anesthesia, intubated the simulated patient's trachea, cared for the simulated patient throughout a diagnostic shoulder arthroscopy that progressed into a Bankart procedure, and extubated the simulated patient following skin closure and emergence. After completing the experiment, subjects completed a NASA-TLX workload probe and a short questionnaire about the drug display's utility3.

In the presence of the drug display, wake-up times were reduced without any instances of tachycardia or hypertension due to insufficient analgesia. The average time from skin closure to the opening

of the patient's eyes was 7.5±2.1 (mean ±SD) minutes in the control condition and 4.5±3.3 minutes in the drug display condition ($p<0.05$). For the NASA-TLX, participants in the display condition reported a decrease in mental demand, effort, and frustration (all $p<0.05$) while perceiving improved performance ($p<0.05$). Their responses to the post-study survey suggested that the drug display might have more utility than other state-of-the-art technologies such as BISÒ and non-invasive cardiac output.

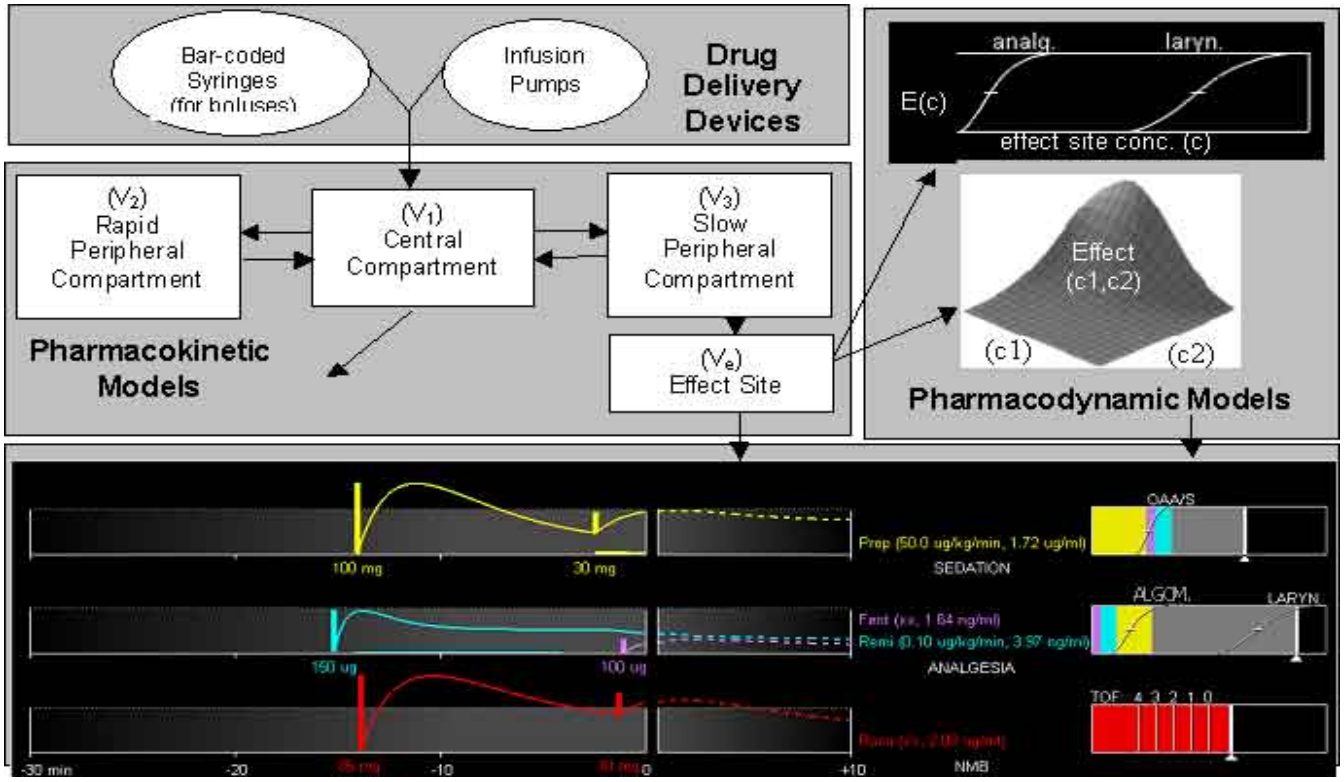


Figure 1: Bar coded syringes and monitored infusion pumps tracks the drugs administered by the physician. Based on the drug input, software programmed PKPD models for remifentanil, propofol and rocuronium predict the effect site concentrations and the drug effects in real time. The left portion of the display shows the dosing history and the PK predictions of past, current and future effect site concentrations. Vertical color-coded bars indicate bolus doses. Infusion rates are displayed as horizontal bars and text. In the center, predicted effect site concentrations are shown 10 minutes into the future. To the right side of the display, three concentration-effect graphs show the current PD model predictions of sedation, analgesia and neuromuscular blockade. Colored bands indicate the effects of individual drugs. The gray bars indicate the synergism of the drugs in combination. The PD effect scales are calibrated to clinical benchmarks such as the OAA/S scale and response to laryngoscopy.

ISSUES:

The results showing a significant improvement in patient wake-up times, the reduction in cognitive workload, and a ranking of this technology comparable with other state-of-the-art anesthesia technologies suggest that use of the display improves clinical decision making and has high utility.

This evaluation in a realistic simulator tested the performance of the display under limited controlled conditions when the predicted pharmacokinetics and dynamics exactly matched the simulated patient's responses. In the operating room, however, individual patient physiological responses may deviate from the responses predicted by population-based models, and clinical conditions can vary tremendously. Thus, clinical assessment in the operating room is required to demonstrate the drug display's clinical utility.

PI: WHITE, STEPHEN H.
University of California at Irvine
Deptartment of Physiology and Biophysics
Med. Sciences I, D346
Irvine, CA 92697-4560
T: (949) 824-7122
blanco@helium.biomol.uci.edu

PROJECT TITLE: Cold Neutrons for Biology and Technology

PARTNERS' NAMES AND AFFILIATIONS:
National Institute of Standards and Technology
Rice University
Carnegie Mellon University
Duke University
University of Pennsylvania

GRANTING NIH INSTITUTE/CENTER: National Center for Research Resources (NCRR)

ABSTRACT:
The Cold Neutrons for Biology and Technology (CNBT) partnership consists of investigators from six universities, the National Institute of Standards and Technology (NIST), Los Alamos National Laboratory (LANL), and the NIH committed to the development of advanced neutron scattering instruments for studies of membrane systems at the NIST Center for Neutron Research (NCNR). Specifically, these instruments will be devoted to basic and applied studies of membranes and macromolecules in membranes, and to membrane-based technologies that include studies of protein complexes with relevance to bioengineering. The instruments, consisting of a fully dedicated biological advanced neutron diffractometer/reflectometer (AND/R) and a 30-meter small-angle neutron spectrometer (SANS) dedicated 10% to biology, will provide combined advantages and capabilities not currently available in the United States. During the first two years of the project, the AND/R, which has already been designed with the aid of a planning grant from the NSF, will be constructed and commissioned and an existing world-class SANS instrument will be optimized for membrane research. At the same time, a high-performance computer system will be put in place to support the concerted use of neutron diffraction and molecular dynamics methods in order to deduce 3-D structural information from 1- or 2-D diffraction data. Finally, new laboratory space adjacent to the neutron instrument hall will be renovated and equipped to serve the special needs of the partnership and the other biological users. Concomitantly, research and technical staff will be recruited. Some early progress on the tasks of the partnership will be achieved using the existing non-optimized SANS and the existing reflection/diffraction instruments at the NCNR during these two years.

The development of the new membrane-optimized instruments will be driven by distinct experiments inspired by the research programs of the CNBT team. The expertise of the team members, drawn from departments of chemistry, physiology, cell biology, and physics, includes membrane diffraction, small angle neutron scattering, membrane molecular dynamics (MD), biosensors, and biomaterials. Linking neutron diffraction measurements to MD simulations of biomolecular structure is an important objective of the team. We foresee a future when computer simulations will allow three-dimensional detail to be inferred routinely from 1- and 2-dimensional neutron and X-ray data.

STATUS OF RESEARCH AND PARTNERSHIP:
The project is young! We are presently working on five major tasks in order to launch the project. (1) Recruitment of a director for the AND/R. (2) Setting up a memorandum of understanding (MOU) between NIST and UCI to enable construction of the AND/R using grant funds. (3) Getting the construction project into the work stream of the engineers at NIST. (4) Ordering parts, making working drawings, and assigning tasks to the instrument shops. (5) Planning for the purchase of high-performance computers at NIST and at UCI. The partnership is generally in fine shape.

ISSUES:
Nothing serious at present. Setting up the mechanism for moving money to NIST for purchasing equipment and services was somewhat difficult. But all problems have been resolved.